# EXHIBIT 32

From: Ghassan Saed gsaed@med.wayne.edu

Subject: Re: SRI abstract Date: May 10, 2021 at 4:10 PM

To: Robert Morris rmorris@med.wayne.edu

Cc: Ghassan Saed gsaed@med.wayne.edu, Amy Harper aharper4@med.wayne.edu



On May 9, 2021, at 9:42 PM, Robert Morris <morris@med.wayne.edu> wrote:

Nice work Ghassan.

Bob

Sent from my iPhone so excuse the brevity and typos.

On May 7, 2021, at 4:21 PM, Ghassan Saed <gsaed@med.wayne.edu> wrote:

Dear Bob and Amy

I have submitted an abstract which is part of our ongoing work with talcum powder and ovarian cancer to SRI. I hope it will get accepted.

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PageID: 230638

Please see attached.

Dr. Ghassan M. Saed Associate Professor of Gynecologic Oncology Director of Ovarian Cancer Biology Research Departments of Obstetrics and Gynecology and Oncology

Member of Tumor Biology and Microenvironment Program Karmanos Cancer Institute

Wayne State University School of Medicine

Detroit, MI 48202 (313) 577-5433 Office

(313) 577-1302 Lab

(313) 577-4633 Fax

<SRI-21 abstract.pdf>

Dr. Ghassan M. Saed

Associate Professor of

Gynecologic Oncology

Director of Ovarian Cancer Biology Research

Departments of Obstetrics and Gynecology and Oncology

Member of Tumor Biology and Microenvironment Program

Karmanos Cancer Institute

Wayne State University School of Medicine

Detroit, MI 48202

(313) 577-5433 Office

(313) 577-1302 Lab

(313) 577-4633 Fax

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**Activity:** Late Breaking Abstract

Current Date/Time: 5/7/2021 3:18:02 PM

Talcum powder induces malignant transformation of human primary normal ovarian epithelial cells

Author Block: Ghassan M. Saed\*, Amy Harper†, Robert Morris\*. Wayne State University, Detroit, MI, United States.

#### Abstract:

Introduction: Molecular and epidemiological studies have demonstrated an association between the genital use of talcum powder and an increased risk of ovarian cancer (OC). Previously we have shown that using an agar transformation assay. The objective of this study is to confirm such an important finding with a different assay.

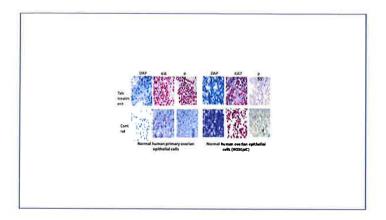
**Methods:** Human primary ovarian epithelial cells (HPOE) and ovarian epithelial cells (HOSEpiC) were treated with either 100 ug/ml of talcum powder or titanium dioxide (TiO2) as a particulate control for 72 hours before assessment of p53 and Ki67 expression with immunohistochemistry (IHC).

**Results:** Focal p53 nuclear staining indicating wild type p53 expression was observed in both cell lines before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear staining was observed indicating p53 mutated form. Additionally, talcum powder treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both cell lines (Figure 1).

**Conclusion:** Exposure to talcum powder induces malignant transformation in ovarian epithelial cells. These findings represent a direct effect of talcum powder exposure and further supports previous studies demonstrating a link between the genital use of talcum powder and increased risk of OC.

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Category (Complete): 06.1-Cancer Biology

Questionnaire (Complete):

Has this abstract been previously presented as it is written?: No

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My submitted abstract(s) contains original data, written in standard scientific form, complete with numeric values and statistical analyses when appropriate.: Yes

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If my abstract contains microarray data, all analyses must be accompanied by confirmation of expression changes with either transcript or protein data.: Not **Applicable** 

All data derived using the same paradigm (set of patients or experiments) will not be separated into multiple abstracts.: Not Applicable

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I will comply with the SRI Withdrawal Policy. : True

Translational Value: Yes

Please describe the translational relevance below: the fact that genital talcum powder use is linked to ovarian cancer risk

Keyword (Complete): ovarian cancer; Talcum powder; immunohistochemistry

Payment (Complete): Your credit card order has been processed on Friday 7 May 2021 at

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# **Background**

Molecular and epidemiological studies have demonstrated an association between the genital use of talcum powder and an increased risk of ovarian cancer (1-4). Several in vitro studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder (1-4). In support of these findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer (5). More importantly, we have recently shown that exposure of normal ovarian epithelial cells to talcum powder induced transformation of these cells, using an agar transformation assay.

## **Objective**

We sought to confirm that exposure of normal ovarian epithelial cells to talcum powder induces transformation of these cells with a different assay.

#### Methods

Human primary ovarian epithelial cells (HPOE) and ovarian epithelial cells (HOSEpiC) were treated with either 100 ug/ml of talcum powder or titanium dioxide (TiO2) as a particulate control for 72 hours before assessment of p53 and Ki67 expression with immunohistochemistry (IHC).

#### Results

Focal p53 nuclear staining indicating wild type p53 expression was observed in both cell lines before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear staining was observed indicating p53 mutated form. Additionally, talcum powder treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both cell lines (Figure 1).

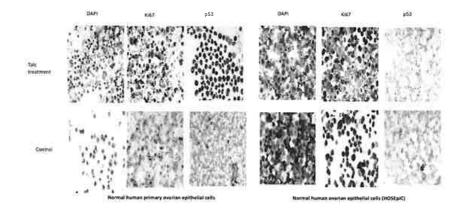


Figure 1: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides were reviewed by two pathologists.

Exposure to t transformation findings represe exposure and demonstrating talcum powde cancer.

1. H. Langseth, S. Perineal use of ta Community Health 2. W. J. Henderson and carcinoma of Commonw 78, 266-3. D. S. Heller, relationship betwee particle burden. Am 4. J. E. Muscat, M. cancer, a critical rev 5, N. M. Fletcher et of Talcum Powder Reprod Sci 26, 160:

Dr. Saed has se witness for the plant remaining author: report.

From: International Journal of Gynecological Cancer onbehalfof@manuscriptcentral.com

Subject: IJGC Decision

Date: March 2, 2021 at 9:47 AM To: gsaed@med.wayne.edu

Cc: aharper4@med.wayne.edu, xin.wang7@wayne.edu, florrie1522@gmail.com, nfletche@med.wayne.edu,

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rmorris@med.wayne.edu, gsaed@med.wayne.edu

[EXTERNAL] -----

COVID-19: A message from BMJ: https://authors.bmj.com/policies/covid-19

Manuscript Number: ijgc-2021-002562

Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

Dear Dr. Saed,

Thank you for submitting your manuscript to International Journal of Gynecological Cancer. The Editorial Team has reviewed your recent submission and, unfortunately, your submission is not considered acceptable for publication.

The journal receives many submissions and due to space limitations are only able to accept a small portion of them. The specific comments of the Editorial Team are included below. Please note that all decisions are final and that the journal does not accept rebuttals.

On initial evaluation of the manuscript, the Editorial team considers that the manuscript addresses a topic viewed outside the scope of the journal and thus, unfortunately, the manuscript will not be considered for further review. Although the manuscript may be considered of related interest, we have collectively determined that the subject addressed in such manuscript is not appropriate for the readership of our journal.

Thank you for submitting your manuscript to the International Journal of Gynecological Cancer. We would be happy to review your future work.

With Kind Regards, Dr. Pedro T. Ramirez Editor-in-Chief International Journal of Gynecological Cancer

Dear sir/madam,

Please consider our manuscript "Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells" for publication in your journal. We have previously published that Talcum powder induces an inflammatory/oxidative stress profile in normal epithelial ovarian cells similar to that seen in ovarian cancer cells. Here we are excited to demonstrate that exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding is intriguing, and work is currently ongoing in our laboratory to understand the mechanism.

Dr. Ghassan M. Saed
Associate Professor of Gynecologic Oncology
Director of Ovarian Cancer Biology Research
Departments of Obstetrics and Gynecology and Oncology
Member of Tumor Biology and Microenvironment Program
Karmanos Cancer Institute
Wayne State University School of Medicine
Detroit, MI 48202
(313) 577-5433 Office
(313) 577-1302 Lab
(313) 577-4633 Fax

1 Talcum powder induces malignant transformation in normal human primary

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2 ovarian epithelial cells

- 4 Amy K. Harper, M.D.<sup>1,2</sup>, Xin Wang, M.S.<sup>1</sup>, Rong Fan, M.S.<sup>1</sup>, Nicole M. Fletcher, Ph.D.<sup>1</sup>,
- Robert T. Morris, M.D.2, \*Ghassan M. Saed, Ph.D.1,2 5
- 6 <sup>1</sup> Department of Obstetrics and Gynecology, <sup>2</sup>Department of Gynecologic Oncology,
- 7 Karmanos Cancer Institute, Detroit, MI, 48201

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- 9 \*Corresponding Author:
- 10 Ghassan M. Saed, Ph.D.
- 11 Associate Professor of Gynecologic Oncology
- 12 Director of Ovarian Cancer Biology Research
- 13 Departments of Obstetrics and Gynecology and Oncology
- 14 Member of Tumor Biology and Microenvironment Program
- 15 Karmanos Cancer Institute
- 16 Wayne State University School of Medicine
- 17 Detroit, MI 48201
- 18 (313) 577-5433 Office phone, (313) 577-8544 Office fax, (313) 577-1302 Lab phone
- 19 gsaed@med.wayne.edu

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24	<b>Abstra</b>	ct
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25 Background: Several studies have linked perineal use of talcum powder to increased 26 risk of ovarian cancer (OC). Here, we determined that exposure to talcum powder induces 27 malignant transformation in human normal ovarian cells. 28 Methods: Human primary ovarian epithelial cells (HPOE), ovarian epithelial cells 29 (HOSEpiC), and primary fibroblasts (NF) were treated with either 100 or 500 ug/ml of 30 talcum powder or titanium dioxide (TiO2) as a particulate control for 72 hours before 31 assessment with a cell transformation assay and p53 and Ki67 immunohistochemistry. 32 **Results:** Treatment with talcum powder resulted in formation of colonies, indicating cell 33 malignant transformation in a dose dependent manner in ovarian cell lines. No colonies 34 formed in the untreated ovarian cells or control ovarian cells (TiO<sub>2</sub> treated) at either dose. 35 There were no colonies formed in talc treated NF cells. Transformed ovarian cells were 36 increased by 11% and 20% in HPOE and 24% and 40% in HOSEpic cells for talcum 37 powder 100 and 500 ug/ml doses, respectively (p<0.05). There were no detectible 38 transformed cells when cells were treated with TiO<sub>2</sub>. Importantly, p53 mutant type as well 39 as increased expression of Ki67 were detected in HPOE and HOSEpic cells when 40 exposed to talcum powder. 41 Conclusion: Exposure to talcum powder induces malignant transformation in ovarian 42 epithelial cells but not in NF cells. These findings represent a direct effect of talcum 43 powder exposure that is specific to normal ovarian cells and further supports previous 44 studies demonstrating an association between the genital use of talcum powder and an 45 increased risk of OC.

46

#### Introduction

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48 Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among 49 women in United States [1]. Epithelial ovarian cancer (EOC) presents with various 50 histopathology, molecular biology, and clinical outcome and is therefore considered a 51 heterogeneous disease [2]. The pathogenesis of EOC is strongly associated with 52 oxidative stress and inflammation [3-5]. 53 Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that has been 54 demonstrated in vitro and is also enhanced in chemoresistant EOC cells [3, 4]. 55 Attenuation of the pro-oxidant state with antioxidants/scavengers has been shown in vitro 56 to selectively induce apoptosis in EOC cells indicating a potential therapeutic value [6, 7]. 57 Talcum Powder has been shown to induce oxidative stress and cell proliferation and to 58 decrease apoptosis in normal ovarian cells and thus may play an important role in the 59 pathogenesis of EOC [8]. 60 The association between genital use of talcum powder and risk of ovarian cancer have 61 been described in numerous studies [8-11]. Several meta-analyses have demonstrated 62 a statistically significant increased risk of ovarian cancer with the genital use of talcum 63 powder [11-13]. In addition, several animal studies have reported that talcum powder 64 causes inflammation and oxidative stress [14-16]. Several in vitro studies have 65 demonstrated a biologic effect when cells in culture are exposed to talcum powder [17-21]. 66 67 In support of these previous findings, we have recently delineated the molecular basis of 68 the association of talcum powder use with increased risk of ovarian cancer [8]. The 69 specific mechanism by which talcum powder exposure causes ovarian cancer has not

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70 been definitively established. Here we clearly demonstrate that exposure to talcum 71 powder induces malignant transformation in human primary normal ovarian epithelial cells 72 and thus, providing a mechanism for the increased risk of ovarian cancer with the genital 73 use of talcum powder.

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#### Methods

Normal human primary ovarian epithelial cells (HPOE): Cells were received at 76 passage 3 (Cell Biologics, Chicago, IL) cryo-preserved in vials containing at least 77 78 0.5x106 cells per ml. Cells were grown in gelatin pre-coated T25 flasks for 2 min and 79 incubated in Cell Biologics' Culture Complete Growth Medium. Cells were expanded for 2-4 passages at a split ratio of 1:2 under the cell culture conditions as specified by Cell 80 Biologics. Human Epithelial Cell Medium is a complete medium designed for the culture 81 of human epithelial cells. It was tested and optimized with epithelial cell growth and 82 proliferation in vitro. Cells were incubated at 37 °C with 5% CO2 and 95% air. 83 84 Normal human ovarian epithelial cells (HOSEpiC): Cells were purchased from ScienCell Research Laboratories, Inc, Carlsbad, California. HOSEpiC cells were isolated 85 from human ovary. HOSEpiC cells were received cryopreserved at passage one in frozen 86 vials, each vial contains 5 x 10<sup>5</sup> cells in 1 ml volume. Cells were further expanded for 2-3 87 passages in Ovarian Epithelial Cell Medium (OEpiCM, Cat. #7311). Cells were incubated 88 at 37°C with 5% CO2 and 95% air. 89 Human normal primary peritoneal fibroblasts: This fibroblast cell line has been 90 91 extensively characterized in previous studies [22]. Cells were grown in Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA), with 10 % fetal bovine serum (FBS, 92

93 Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific, Waltham, 94 MA) as we have previously described [22]. Cells were incubated at 37 °C with 5% 95 CO<sub>2</sub> and 95% air. 96 **Talcum powder treatment:** Talcum baby powder (Johnson & Johnson, New Brunswick, 97 NJ, #30027477, Lot#13717RA) or control particles, Titanium dioxide (TiO2, Spectrum 98 Chemical Corp, Lot No. 2EB0148) were used to treat cells. Talcum powder or TiO2 99 were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute 100 each with Sonic Dismembrator (Fischer Scientific, Model 100). Stock solutions were 101 filtered through 30 µm nylon mesh filters. No visible loss of material has observed. Cells 102 were seeded in 100 mm Petri cell culture dishes (1 x 106) and were treated 24 hours later 103 in duplicate in a fresh media with 100 or 500 µg/ml of talc or titanium dioxide (TiO2) for 72 104 hours. Control: cells (30K) with media only and Negative control: cells (30K) with media 105 and PBS. No cell death was observed after 72 hours in culture in control or treated cells. 106 Titanium dioxide, a naturally occurring particle, has been classified in humans and 107 animals as biologically inert [19, 23]. Titanium dioxide particles are produced and used 108 as fine (~ 0.1-2.5 µm) and nanosize (<0.1 µm) particles [23]. In this study, we used TiO<sub>2</sub> 109 as a particulate control to exclude the effect of material size. Culture plates were washed 110 several times to remove residual particles and collected by trypsin in fresh media. Cells 111 were counted and their concentration was adjusted with fresh media to 1.5 X 106 cells/ml. 112 113 Cells were now ready to be assessed with cell transformation assay (colorimetric), 114 according to the manufacturer protocol (Abcam-235698, Cambridge, MA). The 100 and 115 500 ug/ml doses were chosen based on our previous studies which showed talcum

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powder to induce changes in redox balance of cells at the molecular level [8]. The
experiments were repeated 3 times with a fresh solution of talcum powder and TiO <sub>2</sub> . This
assay is more stable, faster and more sensitive than the traditional Soft-Agar Assay.
Traditional assays require 3-4 weeks of incubation and inconsistent due to independent
counting. An additional advantage of this assay is it's linear range from 10,000-400,000
cells.
A Cell-dose curve was established as described in the manufacturer's protocol. Briefly,
we used cells (5.34 x $10^5$ cells/ml) were suspended in 1X DMEM/10% FBS medium. Cells
were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was
performed using an 8 channel multi pipette by adding 150 µl of media to each well of a
96 well microplate. A 150 $\mu$ l aliquot of the 5.34 x10 $^5$ cells/ml (80 x10 $^3$ cells) was added to
the wells of the first duplicate row. A 150 µl aliquot from the first duplicate row was
removed and added to the next well and mixed. The process was repeated until the seven
serial dilutions were obtained. The final well was blank with media only and no cells. A 35
$\mu l$ aliquot of 1X DMEM/10% FBS and 15 $\mu l$ of WST working solution were then added into
each well and incubate at 37° C for 4 hours. The absorbance was measured by a
microtiter plate reader at 450 nm (Figure 1).
Agarose and WST working solutions were prepared as described in the kit information
sheet (Abcam-235698, Cambridge, MA). The base agarose mix was added into the
required wells in a 96 well plate and kept for 15 minutes at 4°C to solidify the agarose. A

top agarose layer stock solution was prepared by using talcum powder or TiO2 treated

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stock cell solution of 1.5 X 106 cells/ml (30,000 cells per well, which is within the recommended range of the assay) in 1X DMEM/10% FBS medium. The agarose-cell mix was added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10 min at 37°C, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

Immunohistochemistry (IHC) staining and scoring: The IHC panel consisted of antibodies against p53 and Ki-67. The primary antibodies, suppliers, and staining conditions are listed below.

153	Antibody	Clone	Source	Detection System	Dilution
154	P53	DO-7	Ventana	Ventana ultraView DAB	1:500
155	Ki-67	Mib1	Ventana	Ventana ultraView DAB	1:2000

Cytospin slides were prepared from cells and stained using immunoperoxidase labeling performed with the automated XT iVIEW DAB V.1 procedure on the Ultra BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone DO-7 prediluted, Ventana). Antigen retrieval was carried out with CC1, pH 8.0 (Ventana). Sections were incubated with primary antibodies for 36 min at 37°C. All slides were reviewed by two pathologists (Drs Ali and Alrajjal). Cases with discordant Ki-67 estimated results underwent a consensus review at a double-headed microscope. Diffuse "in-block" nuclear staining or

complete negative staining with p53 was considered a positive reaction indicating
mutated p53 status. Focal nuclear staining is consistent with "wild type" p53 and
considered negative. The Proliferation Index (PI) was assessed qualitatively using Ki-67-
stained slides and classified as high PI (>50% positive cells) or low PI (<50% positive
cell).
Statistical Analysis: We performed ANOVAs with Tukey post hoc tests to evaluate the
difference between the three groups (no treatment control, talcum powder treatment and
TiO2 treatment). The values were expressed as mean $\pm$ standard deviation. We used
SPSS v24 for Windows (SPSS, Chicago, Illinois); a p<0.05 defined significance.
Results
Results  Treatment with talcum powder significantly increased the number of transformed normal
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Treatment with talcum powder significantly increased the number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses,
Treatment with talcum powder significantly increased the number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Likewise, but to a greater extent, treatment with talcum
Treatment with talcum powder significantly increased the number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Likewise, but to a greater extent, treatment with talcum powder significantly increased the number of transformed HOSEpiC cells by 24% and

control and the two doses of TiO2 treatment control group (Figure 2, p>0.05).

It is known that cancer cells are able to grow in culture without the need for matrix

attachment. Treatment with talcum powder resulted in formation of colonies, indicating

cell malignant transformation in normal epithelial ovarian cell lines in a dose dependent

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manner (Figure 3). There were no colonies formed in talcum powder treated normal
fibroblasts (Figure 3). There were no colonies formed in either untreated ovarian cells or
control ovarian cells at either dose. There were no detectible transformed cells when cells
were treated with the particulate control, TiO <sub>2</sub> .
To confirm malignant cell transformation observed with the cell transformation assay used
in this study we performed IHC on the normal human primary ovarian epithelial (HPOE)
and normal human ovarian epithelial cells (HOSEpiC) cells staining for p53 and Ki67.
Focal p53 nuclear staining indicating wild type p53 expression was observed in cells
before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours,
diffused "in-block" nuclear staining was observed indicating p53 mutated form (Figure 4).
Additionally, talcum powder treatment increased the proliferation index (PI) in both cell
lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The
PI was significantly increased to 90% in both cell lines (Figure 4).
Discussion
This is the first study to directly show that exposure to talcum powder induces malignant
transformation in ovarian epithelial cells. The ability of talcum powder exposure to induce
transformation appears to be specific to ovarian cells as it did not induce transformation
in peritoneal fibroblasts. (Figure 3).
The link between talcum powder exposure and ovarian cancer have been supported by

the harmful biological effects reported in various cell culture studies [8, 14, 17-21, 24, 25].

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Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer [14, 18]. Macrophages exposed to nano-talc manifested increased levels in inflammatory markers, TNF-alpha, IL-1beta and IL-6 as well as constituent phosphorylation of both p38 and ERK1/2 pathways [18]. p38 MAPK signaling pathway are known to be associated with cisplatin-resistant ovarian cancer [26]. Exposure of macrophages to talc and estradiol has led to increased production of reactive oxygen species and changes in expression of macrophage genes that play a role in cancer development and immunosurveillance [24]. These studies have also shown that ovarian cancer cells were present in larger numbers after co-culture with macrophages exposed to talc powder when in the presence of estradiol [24].

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Oxidative stress have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes in EOC tissues and cells as compared to normal cells [27]. Talcum powder exposure was shown to induce molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer [3, 8]. In all talc-treated cells, there was a significant dose-dependent increase in key prooxidants with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory balance involves the induction of specific mutations in key oxidant and antioxidant enzymes that correlate with alterations in their activities. [8].

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Ovarian cancer cells was shown to manifest increased cell proliferation and decreased
apoptosis, a hallmark of malignant cells, as compared to normal ovarian cells [27].
Indeed, talcum powder further enhanced cell proliferation and inhibited apoptosis in EOC
cells, but more importantly in normal ovarian cells, suggesting talc is a stimulus to the
development of the oncogenic phenotype [8]. Furthermore, CA-125, a membrane-bound
and secreted protein, has been established as a biomarker for disease progression and
response to ovarian cancer treatment [28]. CA-125 was significantly increased to values
approaching clinical significance (35 U/ml in postmenopausal women) in talc treated
normal ovarian cells [8, 28]. Thus, these findings confirmed the inflammatory/redox stress
effects of talcum powder exposure to normal ovarian cells and indicated that this stress
is a key mechanism in the malignant transformation of these cells.

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The dose and time of talcum powder exposure in cell culture experiments used in this study was based on previous studies [8]. These doses are not intended to represent a typical dose when applied to the genital area in women over time. Despite this limitation, the development and use of in vitro models has been valuable in the advancement of research and knowledge on cancer pathogenesis [29]. The cellular transformation demonstrated in this study was significant and informative.

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Anchorage-independent growth is one of the hallmarks of cell transformation and is accepted to be the most accurate and stringent in vitro assay for detecting malignant transformation of cells [30, 31]. The soft agar colony formation assay used in this study is widely accepted and used to evaluate cellular transformation [30, 31]. The Cell

Transformation Assay Kit is faster, stable, more sensitive, and has a wide linear range
(10,000-400,000) cells than the traditional Soft-Agar Assay. Therefore, in this study we
used 30,000 of talcum powder and TiO <sub>2</sub> treated cells as well as control cells to stay within
the recommended number of cells. The assay utilizes the conversion of tetrazolium salt
to formazan by mitochondrial dehydrogenases which is directly proportional to the
number of living transformed cells (Figure 2).
Tumor suppressor p53 gene mutations are frequently seen in ovarian cancers and can
be used as a biomarker to differentiate low from high grade serous ovarian carcinomas.
The methods used for the assessment of p53 (mutant vs. wild type) and Ki67 expression
in this study is identical to the methods used in clinical pathology laboratories for the
diagnosis of the different subtypes of ovarian cancer. The slides were scored and
interpreted independently by two pathologists. Mutant p53 along with increased Ki67
expression were detected in both HPOE and (HOSEpiC) ovarian cells treated with 100
ug/ml talcum powder for 72 hours (Figure 4). These findings supported the malignant
transformation of normal ovarian cells seen in the agar transformation assay (Figure 3).
This study clearly demonstrate that talcum powder exposure induced malignant
transformation of normal ovarian cells in culture which adds to the strong evidence of a
causal relationship between the genital use of talcum powder and ovarian cancer.
Therefore, we consider that future studies should aim to evaluate this finding utilizing
animal models.

277	ln (	conclusion, the ability of talcum powder exposure to induce malignant transformation	
278	ap	pears to be specific to ovarian cells as it did not induce transformation in normal	
279	peritoneal fibroblasts. Further investigation to understand this specific effect of talcum		
280	po	wder on the ovaries is needed.	
281			
282	Ac	knowledgment: We acknowledge Dr. Ruba Ali-Fahmi and Dr. Ahmad Alrajjal from the	
283	department of pathology who helped with immunohistochemistry of p53 and Ki67.		
284			
285	Re	ferences	
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371	Figure Legends
372	
373	Figure 1: Human primary normal ovarian epithelial cell-dose curve. The cell dose curve
374	was established as described in methods using a serial dilution of cells.
375	
376	Figure 2: Equal numbers (30K) of human primary normal ovarian epithelial cells (HPOE),
377	human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells
378	(NF) were seeded for the cell transformation assay as described in methods. After 6 days,
379	the cell number were measured. Standard and samples readings were taken 4 hours after
380	adding WST working solution. Control: cells (30K) with media only.
381	
382	Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human
383	ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF)
384	treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture. Colonies of
385	transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase
386	Contrast Microscope with an Axio camera.
387	
388	Figure 4: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian
389	epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides
390	were reviewed by two pathologists. Diffuse nuclear staining or complete negative staining
391	with p53 is considered a positive reaction indicating mutated p53 status were observed
392	in cells treated with talcum powder. Focal nuclear staining is consistent with wild type p53

393	and considered negative was observed in untreated cells (control). An increase in the
394	proliferation index (Ki67) was observes in talcum powder treated cells versus controls.
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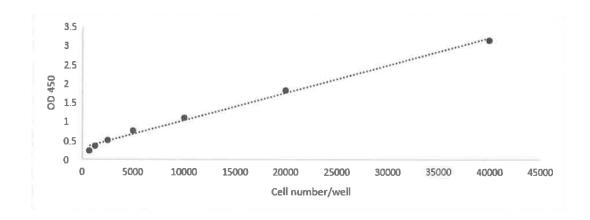
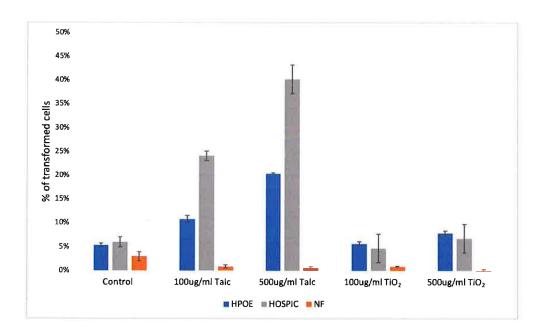


Figure 1: Human primary normal ovarian epithelial cell-dose curve. The cell dose curve was established as described in methods using a serial dilution of cells.



**Figure 2:** Equal numbers (30K) of human primary normal ovarian epithelial cells (HPOE), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution. Control: cells (30K) with media only.

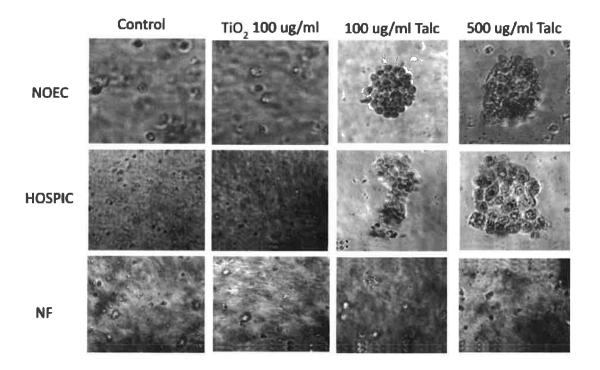


Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

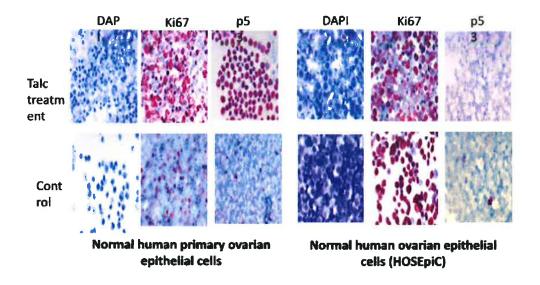


Figure 4: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides were reviewed by two pathologists. Diffuse nuclear staining or complete negative staining with p53 is considered a positive reaction indicating mutated p53 status were observed in cells treated with talcum powder. Focal nuclear staining is consistent with wild type p53 and considered negative was observed in untreated cells (control). An increase in the proliferation index (Ki67) was observes in talcum powder treated cells versus controls.

### **HIGHLIGHTS**

- Several studies have linked perineal use of talcum powder to increased risk of ovarian cancer.
- Several in vitro studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder.
- Exposure to talcum powder induces malignant transformation in human ovarian epithelial cells but not in normal peritoneal fibroblasts.

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Date: February 22, 2021 at 8:14 AM

To: Ghassan M Saed gsaed@med.wayne.edu

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Running title: Talcum powder use and risk of ovarian cancer

Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

Amy K. Harper, M.D.<sup>1,2</sup>, Xin Wang, M.S.<sup>1</sup>, Rong Fan, M.S.<sup>1</sup>, Nicole M. Fletcher, Ph.D.<sup>1</sup>, Robert T. Morris, M.D.<sup>2</sup>, \*Ghassan M. Saed, Ph.D.<sup>1,2</sup>

<sup>1</sup> Department of Obstetrics and Gynecology, <sup>2</sup>Department of Gynecologic Oncology, Karmanos Cancer Institute, Detroit, MI, 48201

# \*Corresponding Author:

Ghassan M. Saed, Ph.D.

Associate Professor of Gynecologic Oncology

Director of Ovarian Cancer Biology Research

Departments of Obstetrics and Gynecology and Oncology

Member of Tumor Biology and Microenvironment Program

Karmanos Cancer Institute

Wayne State University School of Medicine

Detroit, MI 48201

(313) 577-5433 Office phone, (313) 577-8544 Office fax, (313) 577-1302 Lab phone gsaed@med.wayne.edu

PageID: 230681

Capsule: Talcum powder induces malignant transformation in ovarian epithelial cells.

Abstract

Purpose: To study whether exposure to talcum powder induces malignant transformation in

human normal ovarian cells.

**Design:** Prospective experimental study.

Setting: University medical center

Patient(s): Cell lines from normal peritoneum and ovaries

Intervention(s): Human primary ovarian epithelial cells (HPOE), ovarian epithelial cells

(HOSEpiC), and primary fibroblasts (NF) were treated with either 100 or 500 ug/ml of talcum

powder or titanium dioxide (TiO<sub>2</sub>) as a particulate control for 72 hours.

Main outcome measure(s): Transformation of cells was assessed with cell transformation assay

and immunohistochemistry of p53 and Ki67.

Result(s): Treatment with talcum powder resulted in formation of colonies, indicating cell

malignant transformation in a dose dependent manner in ovarian cell lines. No colonies formed

in the untreated ovarian cells or control ovarian cells (TiO<sub>2</sub> treated) at either dose. There were no

colonies formed in talc treated NF cells. Transformed ovarian cells were increased by 11% and

20% in HPOE and 24% and 40% in HOSEpic cells for talcum powder 100 and 500 ug/ml doses,

respectively (p<0.05). There were no detectible transformed cells when cells were treated with

TiO<sub>2</sub>. Importantly, p53 mutant type as well as increased expression of Ki67 were detected in

HPOE and HOSEpic cells when exposed to talcum powder.

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**Conclusion(s):** Exposure to talcum powder induces malignant transformation in ovarian epithelial cells but not in NF cells. These findings represent a direct effect of talcum powder exposure that is specific to normal ovarian cells and further supports previous studies demonstrating an association between the genital use of talcum powder and an increased risk of OC.

**Purpose**: To study whether exposure to talcum powder induces malignant transformation in human normal ovarian cells.

Design: Prospective experimental study.

Setting: University medical center

Patient(s): Cell lines from normal peritoneum and ovaries

**Intervention(s):** Human primary ovarian epithelial cells (HPOE), ovarian epithelial cells (HOSEpiC), and primary fibroblasts (NF) were treated with either 100 or 500 ug/ml of talcum powder or titanium dioxide (TiO<sub>2</sub>) as a particulate control for 72 hours.

**Main outcome measure(s):** Transformation of cells was assessed with cell transformation assay and immunohistochemistry of p53 and Ki67.

Result(s): Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in ovarian cell lines. No colonies formed in the untreated ovarian cells or control ovarian cells (TiO<sub>2</sub> treated) at either dose. There were no colonies formed in talc treated NF cells. Transformed ovarian cells were increased by 11% and 20% in HPOE and 24% and 40% in HOSEpic cells for talcum powder 100 and 500 ug/ml doses, respectively (p<0.05). There were no detectible transformed cells when cells were treated with

TiO<sub>2</sub>. Importantly, p53 mutant type as well as increased expression of Ki67 were detected in HPOE and HOSEpic cells when exposed to talcum powder.

Conclusion(s): Exposure to talcum powder induces malignant transformation in ovarian epithelial cells but not in NF cells. These findings represent a direct effect of talcum powder exposure that is specific to normal ovarian cells and further supports previous studies demonstrating an association between the genital use of talcum powder and an increased risk of OC.

### Introduction

Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States [1]. Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome and is therefore considered a heterogeneous disease [2]. The prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage [3]. This is largely due to the lack of early warning symptoms, screening methods, and the eventual development of chemoresistance [3].

The pathogenesis of EOC is strongly associated with oxidative stress and inflammation [4-8]. Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that has been demonstrated in vitro and is also enhanced in chemoresistant EOC cells [4, 6]. Attenuation of the pro-oxidant state with antioxidants/scavengers has been shown in vitro to selectively induce apoptosis in EOC cells indicating a potential therapeutic value [5, 9, 10]. Talcum Powder has also been shown to induce oxidative stress and cell proliferation and to decrease apoptosis in ovarian cancer cells and in normal ovarian cells [11].

The association between genital use of talcum powder and risk of ovarian cancer have been described in numerous studies [11-18]. Several meta-analyses have demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder [16, 19, 20]. In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress [21-25]. Several *in vitro* studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder [26-31]. In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer [11]. Despite these concerns, the specific mechanism by which talcum powder exposure causes ovarian cancer has not been definitively established.

Here we clearly demonstrate that exposure to talcum powder induces malignant transformation in human primary normal ovarian epithelial cells and thus, providing a mechanism for the increased risk of ovarian cancer with the genital use of talcum powder.

### **Material and Methods**

# Cell lines:

Normal human primary ovarian epithelial cells (HPOE): Cells were purchased from Cell Biologics, Chicago, IL. Cells were received at passage 3 cryo-preserved in vials containing at least  $0.5 \times 10^6$  cells per ml. Cells were grown in gelatin pre-coated T25 flasks for 2 min and incubated in *Cell Biologics'* Culture Complete Growth Medium. Cells were expanded for 2-4 passages at a split ratio of 1:2 under the cell culture conditions as specified by *Cell Biologics*. Human Epithelial Cell Medium is a complete medium designed for the culture of human epithelial cells. It was tested and optimized with epithelial cell growth and proliferation in vitro.

Cells were incubated at 37 °C with 5% CO<sub>2</sub> and 95% air in a humidified incubator. The medium consists of 500 ml of basal medium (containing essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals), supplemented with epithelial cell growth supplement, antibiotics, and fetal bovine serum.

Normal human ovarian epithelial cells (HOSEpiC): Cells were purchased from ScienCell Research Laboratories, Inc, Carlsbad, California. HOSEpiC cells were isolated from human ovary. HOSEpiC cells were received cryopreserved at passage one in frozen vials, each vial contains >5 x 10<sup>5</sup> cells in 1 ml volume. HOSEpiC were further expanded for 2-3 passages under the conditions provided by ScienCell Research Laboratories. Cells were grown in Ovarian Epithelial Cell Medium (OEpiCM, Cat. #7311). Cells were incubated at 37°C with 5% CO<sub>2</sub> and 95% air in a humidified incubator.

Human normal primary peritoneal fibroblasts: Cells were isolated and cultured as we have previously described [32, 33]. The fibroblast cell line has been extensively characterized in previous studies and has been shown to be pure and solely fibroblast cells [32, 33]. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA), supplemented with 10 % fetal bovine serum (FBS, Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific, Waltham, MA) as we have previously described [32, 33]. Cells were incubated at 37 °C with 5% CO<sub>2</sub> and 95% air in a humidified incubator.

**Talcum powder treatment:** Talcum baby powder (Johnson & Johnson, New Brunswick, NJ, #30027477, Lot#13717RA) or control particles, Titanium dioxide (TiO2, Spectrum Chemical Corp, Lot No. 2EB0148) were used to treat cells. Talcum powder or TiO<sub>2</sub> were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each with Sonic Dismembrator (Fischer Scientific, Model 100). Stock solutions were filtered through 30 µm nylon mesh filters. No visible loss of material has observed. Cells were seeded in 100 mm Petri cell culture dishes (1 x 10<sup>6</sup>) and were treated 24 hours later in duplicate in a fresh media with 100 or 500 µg/ml of talc or titanium dioxide (TiO<sub>2</sub>) for 72 hours. Control: cells (30K) with media only and Negative control: cells (30K) with media and PBS. No cell death was observed after 72 hours in culture in control or treated cells. Titanium dioxide, a naturally occurring particle, has been classified in humans and animals as biologically inert [28, 34]. Titanium dioxide particles are produced and used as fine ( $\sim 0.1$ -2.5 µm) and nanosize (< 0.1 µm) particles [34]. In this study, we used TiO<sub>2</sub> as a particulate control to exclude the effect of material size. Culture plates were washed several times to remove residual particles and collected by trypsin in fresh media. Cells were counted and their concentration was adjusted with fresh media to 1.5 X 10<sup>6</sup> cells/ml.

Cells were now ready to be assessed with cell transformation assay (colorimetric), according to the manufacturer protocol (Abcam-235698, Cambridge, MA). The 100 and 500 ug/ml doses were chosen based on our previous studies which showed talcum powder to induce changes in redox balance of cells at the molecular level [11]. The experiments were repeated 3 times with a fresh solution of talcum powder and TiO<sub>2</sub>. This assay is more stable, faster and more sensitive than the traditional Soft-Agar Assay. Traditional assays require 3-4 weeks of incubation and inconsistent due to independent counting. An additional advantage of this assay is it's linear range from 10,000-400,000 cells.

A Cell-dose curve was established as described in the manufacturer's protocol. Briefly, we used cells (5.34 x 10<sup>5</sup> cells/ml) were suspended in 1X DMEM/10% FBS medium. Cells were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was performed

using an 8 channel multi pipette by adding 150 µl of media to each well of a 96 well microplate. A 150 µl aliquot of the 5.34 x10<sup>5</sup> cells/ml (80 x10<sup>3</sup> cells) was added to the wells of the first duplicate row. A 150 µl aliquot from the first duplicate row was removed and added to the next well and mixed. The process was repeated until the seven serial dilutions were obtained. The final well was blank with media only and no cells. A 35 µl aliquot of 1X DMEM/10% FBS and 15 μl of WST working solution were then added into each well and incubate at 37°C for 4 hours. The absorbance was measured by a microtiter plate reader at 450 nm (Figure 1).

Agarose and WST working solutions were prepared as described in the kit information sheet (Abcam-235698, Cambridge, MA). The base agarose mix was added into the required wells in a 96 well plate and kept for 15 minutes at 4°C to solidify the agarose. A top agarose layer stock solution was prepared by using talcum powder or TiO<sub>2</sub> treated stock cell solution of 1.5 X 10<sup>6</sup> cells/ml (30,000 cells per well, which is within the recommended range of the assay) in 1X DMEM/10% FBS medium. The agarose-cell mix was added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10 min at 37°C, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

Immunohistochemistry (IHC) staining and scoring: The IHC panel consisted of antibodies against p53 and Ki-67. The primary antibodies, suppliers, and staining conditions are listed below.

Antibody	Clone	Source Detection System		Dilution	
P53	DO-7	Ventana	Ventana ultraView DAB	1:500	
Ki-67	Mib1	Ventana	Ventana ultraView DAB		1:2000

Cytospin slides were prepared from cells and stained using immunoperoxidase labeling performed with the automated XT iVIEW DAB V.1 procedure on the Ultra BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone DO-7 prediluted, Ventana). Antigen retrieval was carried out with CC1, pH 8.0 (Ventana). Sections were incubated with primary antibodies for 36 min at 37°C. All slides were reviewed by two pathologists (Drs Ali and Alrajjal). Cases with discordant Ki-67 estimated results underwent a consensus review at a double-headed microscope. Diffuse "in-block" nuclear staining or complete negative staining with p53 was considered a positive reaction indicating mutated p53 status. Focal nuclear staining is consistent with "wild type" p53 and considered negative. The Proliferation Index (PI) was assessed qualitatively using Ki-67-stained slides and classified as high PI (>50% positive cells) or low PI (<50% positive cell).

Statistical Analysis: We performed ANOVAs with Tukey post hoc tests to evaluate the difference between the three groups (no treatment control, talcum powder treatment and TiO2 treatment). The values were expressed as mean ± standard deviation. We used SPSS v24 for Windows (SPSS, Chicago, Illinois); a p<0.05 defined significance.

### Results

Treatment with talcum powder significantly increased the number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Likewise, but to a greater extent, treatment with talcum powder significantly increased the number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Talcum powder had no detectable transformation effect on normal peritoneal fibroblasts at either dose (Figure 2). There was no significant difference between the no treatment control and the two doses of TiO2 treatment control group (Figure 2, p>0.05).

It is known that cancer cells are able to grow in culture without the need for matrix attachment. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in normal epithelial ovarian cell lines in a dose dependent manner (Figure 3). There were no colonies formed in talcum powder treated normal fibroblasts (Figure 3). There were no colonies formed in either untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with the particulate control, TiO<sub>2</sub>.

To confirm malignant cell transformation observed with the cell transformation assay used in this study we performed IHC on the normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells staining for p53 and Ki67. Focal p53 nuclear staining indicating wild type p53 expression was observed in cells before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear

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staining was observed indicating p53 mutated form (Figure 4). Additionally, talcum powder

treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and

HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both

cell lines (Figure 4).

Discussion

This is the first study to directly show that exposure to talcum powder induces

transformation in normal human ovarian epithelial cells. The ability of talcum powder exposure

to induce transformation appears to be specific to ovarian cells as it did not induce

transformation in human normal peritoneal fibroblasts. (Figure 3). The reason for this specific

effect of talcum powder on the ovaries in still under investigation.

The dose and time of talcum powder exposure in cell culture experiments used in this

study was based on previous studies [11]. These doses are not intended to represent a typical

dose when applied to the genital area in women over time. Despite this limitation, the

development and use of in vitro models has been valuable in the advancement of research and

knowledge on cancer pathogenesis [35]. The cellular transformation demonstrated in this study

was significant and informative.

The soft agar in vitro colony formation assay is widely accepted and used to evaluate

cellular transformation [36, 37]. Anchorage-independent growth is one of the hallmarks of cell

transformation and is accepted to be the most accurate and stringent in vitro assay for detecting

malignant transformation of cells [36, 37]. In this study we used Cell Transformation Assay Kit,

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which is faster, stable, and more sensitive than the traditional Soft-Agar Assay. The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells. Therefore, in this study we used 30,000 of talcum powder and TiO<sub>2</sub> treated cells as well as control cells to stay within the recommended number of cells. The use of two layers of agar in the 96-well plate allowed the space to utilize such a high number of cells. The assay utilizes the conversion of tetrazolium salt to formazan by mitochondrial dehydrogenases which is directly proportional to the number of living transformed cells (Figure 2).

Tumor suppressor p53 gene mutations are frequently seen in ovarian cancers and can be used as a biomarker to differentiate low from high grade serous ovarian carcinomas. The methods used for the assessment of p53 (mutant vs. wild type) and Ki67 immunohistochemical expression in this study is identical to the methods used in clinical pathology laboratories for the diagnosis of the different subtypes of ovarian cancer. The slides were scored and interpreted independently by two pathologists. Mutant type p53 along with increased Ki67 expression were detected in both normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells treated with 100 ug/ml talcum powder for 72 hours (Figure 4). These findings supported the malignant transformation of normal ovarian cells seen in the agar transformation assay.

The harmful biological effects of link between talcum powder exposure and ovarian cancer have been also confirmed in various in vitro cell culture studies [11, 22, 26-31, 38, 39]. Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer [22, 27]. Macrophages exposed to nano-talc manifested increased levels in inflammatory markers, TNF-alpha, IL-1beta and IL-6 as well as constituent phosphorylation of both p38 and ERK1/2 pathways [27], p38 MAPK signaling pathway are known to be associated with cisplatin-resistant ovarian cancer [40]. Data suggest that nano-talc toxicity on human alveolar basal epithelial cells was mediated through oxidative stress [30]. Exposure of macrophages to talc and estradiol has led to increased production of reactive oxygen species and changes in expression of macrophage genes that play a role in cancer development and immunosurveillance [38]. These studies have also shown that ovarian cancer cells were present in larger numbers after co-culture with macrophages exposed to talc powder when in the presence of estradiol [38].

Oxidative stress and inflammation have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes in EOC tissues and cells as compared to normal cells indicating an enhanced redox state [41]. This redox state is further enhanced in chemoresistant EOC cells as evidenced by a further increase in key pro-oxidant enzymes and a decrease in anti-oxidant levels, suggesting a shift towards a pro-oxidant state [41]. Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian [41].

Our laboratory was the first to confirm the cellular effect of talcum powder and provide a potential molecular mechanism [11]. Talcum powder exposure induced molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer [4, 11]. In all talc-treated cells, there was a significant dose-dependent increase in key prooxidants with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory

balance involves the induction of specific mutations in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes suggests a genetic predisposition to developing ovarian cancer with genital talcum powder use [11].

We have previously reported that EOC cells manifest increased cell proliferation and decreased apoptosis, a hallmark of malignant cells, as compared to normal ovarian epithelial cells [41]. Recently, we have shown that talcum powder further enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype [11]. Furthermore, CA-125, a membrane-bound and secreted protein, has been established as a biomarker for disease progression and response to ovarian cancer treatment [42]. CA-125 expression was significantly increased to values approaching clinical significance (35 U/ml in postmenopausal women) in talc treated human normal epithelial ovarian cells [11, 42]. Collectively, these findings confirmed the inflammatory/redox stress effects of talcum powder exposure to normal ovarian epithelial cells and indicated that this stress is a key mechanism in the malignant transformation of these cells.

The link between genital talcum powder use and ovarian cancer has been shown in numerous epidemiological studies. In addition, the inflammatory effects of talcum powder have been demonstrated in humans, animals, and cells in culture. This study which clearly demonstrates malignant transformation of normal ovarian cells in culture adds to the strong evidence of a causal relationship between the genital use of talcum powder and ovarian cancer.

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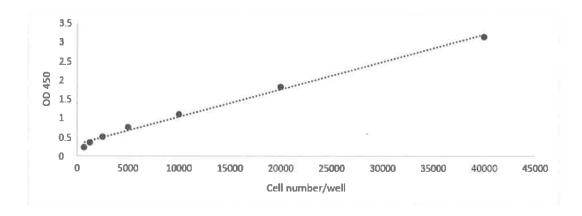


Figure 1: Human primary normal ovarian epithelial cell-dose curve. The cell dose curve was established as described in methods using a serial dilution of cells.

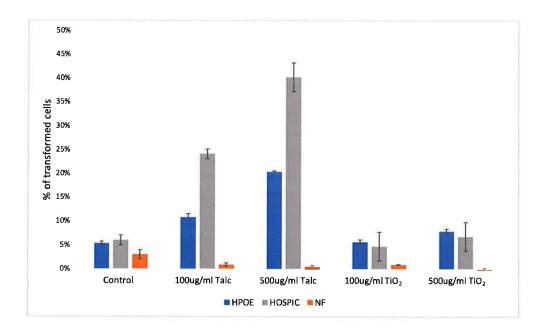
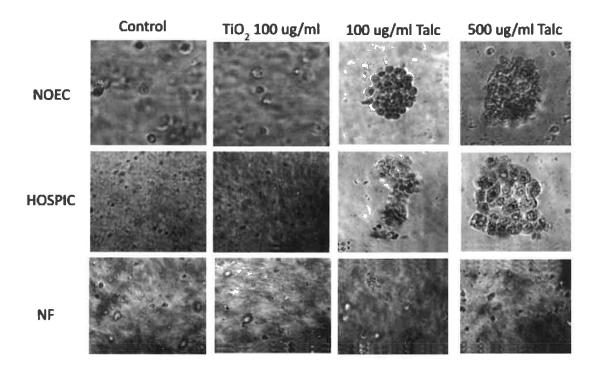
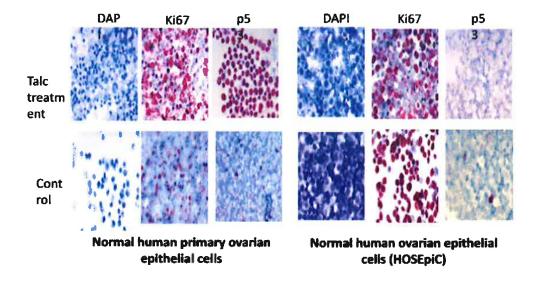


Figure 2: Equal numbers (30K) of human primary normal ovarian epithelial cells (HPOE), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution. Control: cells (30K) with media only.



**Figure 3:** Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.



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Figure 4: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides were reviewed by two pathologists. Diffuse nuclear staining or complete negative staining with p53 is considered a positive reaction indicating mutated p53 status were observed in cells treated with talcum powder. Focal nuclear staining is consistent with wild type p53 and considered negative was observed in untreated cells (control). An increase in the proliferation index (Ki67) was observes in talcum powder treated cells versus controls.

Running title: Talcum powder use and risk of ovarian cancer

Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

PageID: 230704

Amy K. Harper, M.D.<sup>1,2</sup>, Xin Wang, M.S.<sup>1</sup>, Rong Fan, M.S.<sup>1</sup>, Nicole M. Fletcher, Ph.D.<sup>1</sup>, Robert T. Morris, M.D.<sup>2</sup>, \*Ghassan M. Saed, Ph.D.<sup>1,2</sup>

<sup>1</sup> Department of Obstetrics and Gynecology, <sup>2</sup>Department of Gynecologic Oncology, Karmanos Cancer Institute, Detroit, MI, 48201

# \*Corresponding Author:

Ghassan M. Saed, Ph.D.

Associate Professor of Gynecologic Oncology

Director of Ovarian Cancer Biology Research

Departments of Obstetrics and Gynecology and Oncology

Member of Tumor Biology and Microenvironment Program

Karmanos Cancer Institute

Wayne State University School of Medicine

Detroit, MI 48201

(313) 577-5433 Office phone, (313) 577-8544 Office fax, (313) 577-1302 Lab phone

# gsaed@med.wayne.edu

Key words: talc, talcum powder, titanium dioxide, epithelial ovarian cancer, transformation, primary cells, cell proliferation, colonies

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Declaration of Conflicting Interests: Dr. Saed has served as a paid consultant and expert

witness for the plaintiffs in the talcum powder litigation. The remaining authors have no potential

conflicts of interest to report.

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### **Author contributions:**

Amy Harper: acquisition of data, study conception and design, critical revision

Xin Wang: acquisition of data

Rong Fan: acquisition of data

Nicole M. Fletcher: interpretation of data, study conception and design, critical revision

Robert T. Morris: interpretation of data, critical revision

Ghassan M. Saed: acquisition of data, study conception and design, drafting of manuscript,

critical revision

**Gynecologic Oncology** GYN-20-1870: Final Decision February 2, 2021 at 12:27 AM Ghassan M. Saed



## [EXTERNAL]

Ms. No.: GYN-20-1870

Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

Corresponding Author: Dr. Ghassan M. Saed

Authors: Amy Harper, MD; Xin Wang, MS; rong fan, MS; Nicole Fletcher, Ph.D; Robert Morris, MD

Dear Dr. Saed,

Your paper, referenced above, has now been reviewed by at least two experts in the field and the Editors. Based on the reviewers' comments, we must inform you that while your work is not without merit, we are unable to accept your manuscript for publication in Gynecologic Oncology. In the last year we have seen a significant increase in the number of manuscripts submitted to the Journal and as a result, we are now accepting less than 20% of the manuscripts submitted to the Gynecologic Oncology.

We have attached the comments of the reviewers below in order for you to understand the basis for our decision. We hope that their thoughtful comments will help you in your future studies and possibly, with submission to another journal. Please note that a revised version of the current manuscript should not be submitted for another review to Gynecologic Oncology .

The critique of this paper in no way implies a lack of interest in this area of research, and we invite you to submit your future work to the Journal.

Sincerely,

Barbara A. Goff, MD Gynecologic Oncology

Editorial Office Elsevier E-mail: gyn@elsevier.com

Reviewers' comments:

Reviewer #1: GYN-20-1870

Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

This study examines the effects of talcum powder on normal human primary ovarian surface epithelium. The authors utilize an in vitro anchorage-independent growth assay to assess transformation of ovarian surface epithelial cells in response to talcum powder and follow this with immunohistochemical markers for proliferative index (Ki-67) and p53 expression patterns. The authors identified an increase in transformed cell percentage with increasing concentration of talcum powder, and correlated these findings with observed increases in Ki-67 proliferative index and abnormal p53 staining. The authors conclude that talcum powder induces malignant transformation of ovarian surface epithelial cells.

As presented, the manuscript presents several major issues that warrant attention prior to publication. Of primary concern is the reliance on a single commercial assay for assessment of transformation that has not been established in the literature. Moreover, appropriate statistical tests were not applied and thus the data are difficult to interpret. The clinical relevance is questionable given the arbitrary dose selection of talcum powder, and perhaps more importantly the examination of ovarian surface epithelial cells without comparison to fallopian tube secretory epithelium. Given that the prevailing evidence suggests the origin of high-grade serous ovarian cancer is the fallopian tube, the data presented are of limited relevance. Further demonstration of transformational changes is required before resubmission.

#### Specific Comments:

- It is now generally accepted that most high-grade serous ovarian carcinomas arise from the fallopian tube (FT) fimbrial epithelium. Hence, these studies should examine the effects of talcum powder on FT cells. There are commercial (e.g., ATCC) and academic sources to obtain these cells.
- Further, with the FT fimbria as the dominant site of origin for bigh-grade sergus carcinoma, it is concentually difficult

- to understand why ascending talcum powder would preferentially affect the fimbria and not the more proximal portions of the FT. The authors should address these concepts.
- The reviewer recommends that further work be undertaken to establish whether talcum powder induces functional changes in ovarian epithelial cells suggestive of malignant transformation. The correlation to IHC is insufficient to draw this conclusion, and thus the results of this study are overinterpreted. Given that the transformed cells were not subcultured or further analyzed following treatment conditions to show phenotypic, genetic/epigenetic or functional changes, the changes seen at IHC may be explained by cellular responses to treatments. Whereas durable alterations in p53 staining may indicate mutations, as is the case clinically, p53 expression at a single time point following treatment cannot differentiate between novel mutations and physiologic responses to a given treatment condition. The IHC data would be further strengthened by functional data and/or genomic analysis.
- Statistical analysis: Two major issues arise. The first is the use of paired t-test in an experiment with three treatment conditions (media, Talc, TiO2). This necessitates analysis of variance and multiple comparisons. Additionally, significance in not denoted in figure 2. The authors conclude that proliferative index is elevated to 90%, but do not show evidence or statistical analysis to this effect. Moreover, the methods describe a binary method of scoring Ki-67 of high or low, calling into question how the 90% value was generated.
- Figure 1: While the standard curve establishes the ability of the assay to detect cell number, it offers little to the reading of the manuscript and could be reserved for supplementary data.
- The difference between HPOE and HOSEpiC needs to be mentioned. Are HOSEpiC immortalized in some way?
- Figure 2: Statistical significance is not displayed on the figure, nor are important statistical comparisons between the no treatment control and TiO2
- Figure 3: NOEC 100ug/mL and HOSPIC 500ug/ML Talc panels appear to be identical. TiO2 at 500ug/mL is conspicuously missing.
- Figure 4: Top left panel (DAP/Talc) is identical to DAP/Control. Inadequate white balancing detracts from the reader's ability to appreciate colorimetric differences between the panels.
- Several phrases describing cell culture and colorimetric assay within the methods and discussion appear to be taken verbatim from the manufacturers' websites: examples below.
- Line 80-82: Cells were grown in T25 tissue culture flasks precoated with gelatin-based coating solution for 2 min and incubated in Cell Biologics' Culture Complete Growth Medium which generally took 3-7 days.

From Cell Biologics website: "Human Primary Ovarian Epithelial Cells are grown in T25 tissue culture flasks precoated with gelatin-based coating solution for 2 min and incubated in Cell Biologics' Culture Complete Growth Medium generally for 3-7 days."

Line 126-130: "This assay was chosen because it is more stable, faster and more sensitive than the traditional Soft-Agar Assay that is lengthy (3-4 weeks incubation), laborious (counting colonies) and inconsistent (due to subjective counting). The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells."

From abcam website: "...is lengthy (3-4 weeks incubation), laborious (counting colonies) and inconsistent (due to subjective counting)... The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells."

- Line 185: "Anchorage-independent growth is a hallmark of cancer cells"
  - From abcam website: "Anchorage-independent cell growth is the hallmark of cell transformation"
- Line 223-226: The kit is based on the conversion of the tetrazolium salt (WST) to formazan by cellular mitochondrial dehydrogenases. The generated signal is directly proportional to the number of living cells and thus can accurately determine number of transformed cells.

From abcam website: The kit is based on the conversion of the tetrazolium salt (WST) to formazan by cellular mitochondrial dehydrogenases. The generated signal is directly proportional to the number of living cells.

Reviewer #2: Ultimately these data are too premature for publication, the authors present very preliminary in vitro data suggesting that talcum powder may induce malignant change in normal ovarian epithelial cells, but not in fibroblasts. The data are premature, restricted to two cell lines and really offer no significant mechanistic insight.

I also think the dose of talcum powder is extremely high, I calculate it to be 263mM for the lower dose which is unlikely to ever replicate physological dosing and although the authors recognise this in the discussion it is a major experimental flaw and makes interpretation of results very difficult. The use of IHC to determine p53 mutation status is not very senstiive and I would suggest this needs to be confirmed with sequencing

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/ygyno/login.asp?a=r). Please contact the publication office if you have any questions.

January 04, 2021

To: Editor-in-Chief, Gynecologic Oncology

Please consider our priority report entitled Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells for publication in Gynecologic Oncology. In this study, we determined that exposure to talcum powder induces malignant transformation in human normal ovarian epithelial cells but not in human normal peritoneal fibroblasts. These findings represent a direct effect of talcum powder exposure that is specific to normal ovarian cells and further supports previous studies demonstrating an association between the genital use of talcum powder and an increased risk of ovarian cancer. This study is fitting for Gynecologic Oncology because of the potential ovarian cancer risk that is associated with talcum powder use. The material contained in the manuscript has not been published, has not been submitted, or is not being submitted elsewhere for publication.

## Author contributions:

Amy Harper: acquisition of data, study conception and design, critical revision

Xin Wang: acquisition of data Rong Fan: acquisition of data

Nicole M. Fletcher: acquisition of data, interpretation of data, study conception and design, critical revision

Robert T. Morris: interpretation of data, critical revision

Ghassan M. Saed: study conception and design, drafting of manuscript, critical revision

Sincerely,

Dr. Ghassan M. Saed, Associate Professor

Ifrais - Soed

Director of Ovarian Cancer Biology Research

Departments of Obstetrics and Gynecology

Cell Biology and Anatomy, and Physiology

Member in the Karmanos Cancer Institute

Molecular Biology and Genetics Program

Wayne State University School of Medicine

Detroit, MI 48202

(313) 577-5433 Office, (313) 577-1302 Lab (313) 577-8554 Fax

Talcum powder induces malignant transformation in normal human primary

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- ovarian epithelial cells 2
- Amy K. Harper, M.D.<sup>1,2</sup>, Xin Wang, M.S.<sup>1</sup>, Rong Fan, M.S.<sup>1</sup>, Nicole M. Fletcher, Ph.D.<sup>1</sup>, 3
- Robert T. Morris, M.D.<sup>2</sup>, \*Ghassan M. Saed, Ph.D.<sup>1,2</sup> 4
- 5 <sup>1</sup> Department of Obstetrics and Gynecology, <sup>2</sup>Department of Gynecologic Oncology,
- Karmanos Cancer Institute, Detroit, MI, 48201 6
- Key words: talc, talcum powder, titanium dioxide, epithelial ovarian cancer, 7
- 8 transformation, primary cells, cell proliferation, colonies
- 10 \*Corresponding Author:
- 11 Ghassan M. Saed, Ph.D.
- 12 Associate Professor of Gynecologic Oncology
- 13 Director of Ovarian Cancer Biology Research
- 14 Departments of Obstetrics and Gynecology and Oncology
- 15 Member of Tumor Biology and Microenvironment Program
- 16 Karmanos Cancer Institute
- 17 Wayne State University School of Medicine
- 18 Detroit, MI 48201
- 19 (313) 577-5433 Office phone, (313) 577-8544 Office fax, (313) 577-1302 Lab phone
- 20 gsaed@med.wayne.edu

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**Abstract** 

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25 Objective: Several studies have linked perineal use of talcum powder to increased risk

26 of ovarian cancer (OC). Here, we determined that exposure to talcum powder induces

malignant transformation in human normal ovarian cells.

28 Methods: Human primary ovarian epithelial cells (HPOE), ovarian epithelial cells

29 (HOSEpiC), and primary fibroblasts (NF) were treated with either 100 or 500 ug/ml of

talcum powder or titanium dioxide (TiO2) as a particulate control for 72 hours before

assessment with a cell transformation assay and p53 and Ki67 immunohistochemistry.

32 Results: Treatment with talcum powder resulted in formation of colonies, indicating cell

33 malignant transformation in a dose dependent manner in ovarian cell lines. No colonies

formed in the untreated ovarian cells or control ovarian cells (TiO<sub>2</sub> treated) at either dose.

35 There were no colonies formed in talc treated NF cells. Transformed ovarian cells were

increased by 11% and 20% in HPOE and 24% and 40% in HOSEpic cells for talcum

powder 100 and 500 ug/ml doses, respectively (p<0.05). There were no detectible

transformed cells when cells were treated with TiO<sub>2</sub>. Importantly, p53 mutant type as well

as increased expression of Ki67 were detected in HPOE and HOSEpic cells when

exposed to talcum powder.

41 Conclusion: Exposure to talcum powder induces malignant transformation in ovarian

epithelial cells but not in NF cells. These findings represent a direct effect of talcum

powder exposure that is specific to normal ovarian cells and further supports previous

studies demonstrating an association between the genital use of talcum powder and an

increased risk of OC.

#### Introduction

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Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States [1]. Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome and is therefore considered a heterogeneous disease [2]. The prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage [3]. This is largely due to the lack of early warning symptoms, screening methods, and the eventual development of chemoresistance [3].

The pathogenesis of EOC is strongly associated with oxidative stress and inflammation [4-8]. Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that has been demonstrated in vitro and is also enhanced in chemoresistant EOC cells [4, 6]. Attenuation of the pro-oxidant state with antioxidants/scavengers has been shown in vitro to selectively induce apoptosis in EOC cells indicating a potential therapeutic value [5, 9, 10]. Talcum Powder has also been shown to induce oxidative stress and cell proliferation and to decrease apoptosis in ovarian cancer cells and in normal ovarian cells [11].

The association between genital use of talcum powder and risk of ovarian cancer have been described in numerous studies [11-18]. Several meta-analyses have demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder [16, 19, 20]. In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress [21-25]. Several in vitro studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder [26-31]. In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer [11].

- Despite these concerns, the specific mechanism by which talcum powder exposure 70 71 causes ovarian cancer has not been definitively established.
  - Here we clearly demonstrate that exposure to talcum powder induces malignant transformation in human primary normal ovarian epithelial cells and thus, providing a mechanism for the increased risk of ovarian cancer with the genital use of talcum powder.

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#### **Material and Methods**

#### Cell lines:

- Normal human primary ovarian epithelial cells (HPOE): Cells were purchased from Cell Biologics, Chicago, IL. Cells were received at passage 3 cryo-preserved in vials containing at least 0.5x10<sup>6</sup> cells per ml. Cells were grown in T25 tissue culture flasks precoated with gelatin-based coating solution for 2 min and incubated in Cell Biologics' Culture Complete Growth Medium which generally took 3-7 days. Cells were expanded for 2-4 passages at a split ratio of 1:2 under the cell culture conditions as specified by Cell Biologics. Human Epithelial Cell Medium is a complete medium designed for the culture of human epithelial cells. It was tested and optimized with epithelial cell growth and proliferation in vitro. Cells were incubated at 37 °C with 5% CO<sub>2</sub> and 95% air in a humidified incubator. The medium consists of 500 ml of basal medium (containing essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals), supplemented with epithelial cell growth supplement, antibiotics, and fetal bovine serum.
- 91 Normal human ovarian epithelial cells (HOSEpiC): Cells were purchased from 92 ScienCell Research Laboratories, Inc, Carlsbad, California. HOSEpiC cells were isolated

93 from human ovary. HOSEpiC cells were received cryopreserved at passage one in frozen 94 vials, each vial contains >5 x 10<sup>5</sup> cells in 1 ml volume. HOSEpiC were further expanded 95 for 2-3 passages under the conditions provided by ScienCell Research Laboratories. 96 Cells were grown in Ovarian Epithelial Cell Medium (OEpiCM, Cat. #7311). Cells were 97 incubated at 37°C with 5% CO<sub>2</sub> and 95% air in a humidified incubator. 98 Human normal primary peritoneal fibroblasts: Cells were isolated and cultured as we 99 have previously described [32, 33]. The fibroblast cell line has been extensively 100 characterized in previous studies and has been shown to be pure and solely fibroblast 101 cells [32, 33]. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) 102 (Invitrogen, Carlsbad, CA), supplemented with 10 % fetal bovine serum (FBS, Innovative 103 Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific, Waltham, MA) as we 104 have previously described [32, 33]. Cells were incubated at 37 °C with 5% CO2 and 95% 105 air in a humidified incubator. 106 Talcum powder treatment: Talcum baby powder (Johnson & Johnson, New Brunswick, 107 NJ, #30027477, Lot#13717RA) or control particles, Titanium dioxide (TiO<sub>2</sub>, Spectrum 108 Chemical Corp, Lot No. 2EB0148) were used to treat cells. Talcum powder or TiO<sub>2</sub> 109 were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute 110 each with Sonic Dismembrator (Fischer Scientific, Model 100). Stock solutions were 111 filtered through 30 µm nylon mesh filters. No visible loss of material has observed. Cells 112 were seeded in 100 mm Petri cell culture dishes (1 x 106) and were treated 24 hours later 113 in duplicate in a fresh media with 100 or 500 µg/ml of talc or titanium dioxide (TiO<sub>2</sub>) for 72 114 hours. Control: cells (30K) with media only and Negative control: cells (30K) with media 115 and PBS. No cell death was observed after 72 hours in culture in control or treated cells.

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Titanium dioxide, a naturally occurring particle, has been classified in humans and animals as biologically inert [28, 34]. Titanium dioxide particles are produced and used as fine ( $\sim 0.1-2.5 \,\mu\text{m}$ ) and nanosize ( $< 0.1 \,\mu\text{m}$ ) particles [34]. In this study, we used TiO<sub>2</sub> as a particulate control to exclude the effect of material size. Culture plates were washed several times to remove residual particles and collected by trypsin in fresh media. Cells were counted and their concentration was adjusted with fresh media to 1.5 X 10<sup>6</sup> cells/ml.

Cells were now ready to be assessed with cell transformation assay (colorimetric), according to the manufacturer protocol (Abcam-235698, Cambridge, MA). The 100 and 500 ug/ml doses were chosen based on our previous studies which showed talcum powder to induce changes in redox balance of cells at the molecular level [11]. The experiments were repeated 3 times with a fresh solution of talcum powder and TiO2. This assay was chosen because it is more stable, faster and more sensitive than the traditional Soft-Agar Assay that is lengthy (3-4 weeks incubation), laborious (counting colonies) and inconsistent (due to subjective counting). The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells.

A Cell-dose curve was established as described in the manufacturer's protocol. Briefly, we used cells (5.34 x 10<sup>5</sup> cells/ml) were suspended in 1X DMEM/10% FBS medium. Cells were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was performed using an 8 channel multi pipette by adding 150 µl of media to each well of a 96 well microplate. A 150 μl aliquot of the 5.34 x10<sup>5</sup> cells/ml (80 x10<sup>3</sup> cells) was added to the wells of the first duplicate row. A 150 µl aliquot from the first duplicate row was removed and added to the next well and mixed. The process was repeated until the seven serial dilutions were obtained. The final well was blank with media only and no

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cells. A 35 µl aliquot of 1X DMEM/10% FBS and 15 µl of WST working solution were then added into each well and incubate at 37°C for 4 hours. The absorbance was measured by a microtiter plate reader at 450 nm (Figure 1).

Agarose and WST working solutions were prepared as described in the kit information sheet (Abcam-235698, Cambridge, MA). The base agarose mix was added into the required wells in a 96 well plate and kept for 15 minutes at 4°C to solidify the agarose. A top agarose layer stock solution was prepared by using talcum powder or TiO<sub>2</sub> treated stock cell solution of 1.5 X 106 cells/ml (30,000 cells per well, which is within the recommended range of the assay) in 1X DMEM/10% FBS medium. The agarose-cell mix was added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10 min at 37°C, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

Immunohistochemistry (IHC) staining and scoring: The IHC panel consisted of antibodies against p53 and Ki-67. The primary antibodies, suppliers, and staining conditions are listed below.

160	Antibody	Clone	Source	Detection System	Dilution
161	P53	DO-7	Ventana	Ventana ultraView DAB	1:500
162	Ki-67	Mib1	Ventana	Ventana ultraView DAB	1:2000

Cytospin slides were prepared from cells and stained using immunoperoxidase labeling performed with the automated XT iVIEW DAB V.1 procedure on the Ultra BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone DO-7 prediluted, Ventana). Antigen retrieval was carried out with CC1, pH 8.0 (Ventana). Sections were incubated with primary antibodies for 36 min at 37°C. All slides were reviewed by two pathologists (Drs Ali and Alrajjal). Cases with discordant Ki-67 estimated results underwent a consensus review at a double-headed microscope. Diffuse "in-block" nuclear staining or complete negative staining with p53 was considered a positive reaction indicating mutated p53 status. Focal nuclear staining is consistent with "wild type" p53 and considered negative. The Proliferation Index (PI) was assessed qualitatively using Ki-67-stained slides and classified as high PI (>50% positive cells) or low PI (<50% positive cell).

**Statistical Analysis**: Data was analyzed with paired t-tests. Data are expressed, as means +/- SD. Significance was determined as P <0.05.

#### Results

Treatment with talcum powder significantly increased the number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Likewise, but to a greater extent, treatment with talcum powder significantly increased the number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Talcum powder had no detectable transformation effect on normal peritoneal fibroblasts (Figure 2).

Anchorage-independent growth is a hallmark of cancer cells. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in normal epithelial ovarian cell lines in a dose dependent manner (Figure 3). There were no colonies formed in talcum powder treated normal fibroblasts (Figure 3). There were no colonies formed in either untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with the particulate control, TiO<sub>2</sub>.

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To confirm malignant cell transformation observed with the cell transformation assay used in this study we performed IHC on the normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells staining for p53 and Ki67. Focal p53 nuclear staining indicating wild type p53 expression was observed in cells before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear staining was observed indicating p53 mutated form (Figure 4). Additionally, talcum powder treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both cell lines (Figure 4).

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#### Discussion

This is the first study to directly show that exposure to talcum powder induces transformation in normal human ovarian epithelial cells. The ability of talcum powder exposure to induce transformation appears to be specific to ovarian cells as it did not induce transformation in human normal peritoneal fibroblasts. (Figure 3). The reason for this specific effect of talcum powder on the ovaries in still under investigation.

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The dose and time of talcum powder exposure in cell culture experiments used in this study was based on previous studies [11]. These doses are not intended to represent a typical dose when applied to the genital area in women over time. Despite this limitation, the development and use of in vitro models has been valuable in the advancement of research and knowledge on cancer pathogenesis [35]. The cellular transformation demonstrated in this study was significant and informative.

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The soft agar in vitro colony formation assay is widely accepted and used to evaluate cellular transformation [36, 37]. Anchorage-independent growth is one of the hallmarks of cell transformation and is accepted to be the most accurate and stringent in vitro assay for detecting malignant transformation of cells [36, 37]. In this study we used Cell Transformation Assay Kit, which is faster, stable, and more sensitive than the traditional Soft-Agar Assay. The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells. Therefore, in this study we used 30,000 of talcum powder and TiO<sub>2</sub> treated cells as well as control cells to stay within the recommended number of cells. The use of two layers of agar in the 96-well plate allowed the space to utilize such a high number of cells. The kit is based on the conversion of the tetrazolium salt (WST) to formazan by cellular mitochondrial dehydrogenases. The generated signal is directly proportional to the number of living cells and thus can accurately determine number of transformed cells (Figure 2).

Tumor suppressor p53 gene mutations are frequently seen in ovarian cancers and can be used as a biomarker to differentiate low from high grade serous ovarian carcinomas. The methods used for the assessment of p53 (mutant vs. wild type) and Ki67 immunohistochemical expression in this study is identical to the methods used in clinical

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pathology laboratories for the diagnosis of the different subtypes of ovarian cancer. The slides were scored and interpreted independently by two pathologists. Mutant type p53 along with increased Ki67 expression were detected in both normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells treated with 100 ug/ml talcum powder for 72 hours (Figure 4). These findings supported the malignant transformation of normal ovarian cells seen in the agar transformation assay.

The harmful biological effects of link between talcum powder exposure and ovarian cancer have been also confirmed in various in vitro cell culture studies [11, 22, 26-31, 38, 39]. Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer [22, 27]. Macrophages exposed to nano-talc manifested increased levels in inflammatory markers, TNF-alpha, IL-1beta and IL-6 as well as constituent phosphorylation of both p38 and ERK1/2 pathways [27]. p38 MAPK signaling pathway are known to be associated with cisplatin-resistant ovarian cancer [40]. Data suggest that nano-talc toxicity on human alveolar basal epithelial cells was mediated through oxidative stress [30]. Exposure of macrophages to talc and estradiol has led to increased production of reactive oxygen species and changes in expression of macrophage genes that play a role in cancer development and immunosurveillance [38]. These studies have also shown that ovarian cancer cells were present in larger numbers after co-culture with macrophages exposed to talc powder when in the presence of estradiol [38].

Oxidative stress and inflammation have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes

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in EOC tissues and cells as compared to normal cells indicating an enhanced redox state [41]. This redox state is further enhanced in chemoresistant EOC cells as evidenced by a further increase in key pro-oxidant enzymes and a decrease in anti-oxidant levels, suggesting a shift towards a pro-oxidant state [41]. Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian [41].

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Our laboratory was the first to confirm the cellular effect of talcum powder and provide a potential molecular mechanism [11]. Talcum powder exposure induced molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer [4, 11]. In all talc-treated cells, there was a significant dose-dependent increase in key prooxidants with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory balance involves the induction of specific mutations in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes suggests a genetic predisposition to developing ovarian cancer with genital talcum powder use [11].

We have previously reported that EOC cells manifest increased cell proliferation and decreased apoptosis, a hallmark of malignant cells, as compared to normal ovarian epithelial cells [41]. Recently, we have shown that talcum powder further enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype

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[11]. Furthermore, CA-125, a membrane-bound and secreted protein, has been established as a biomarker for disease progression and response to ovarian cancer treatment [42]. CA-125 expression was significantly increased to values approaching clinical significance (35 U/ml in postmenopausal women) in talc treated human normal epithelial ovarian cells [11, 42]. Collectively, these findings confirmed the inflammatory/redox stress effects of talcum powder exposure to normal ovarian epithelial cells and indicated that this stress is a key mechanism in the malignant transformation of these cells.

The link between genital talcum powder use and ovarian cancer has been shown in numerous epidemiological studies. In addition, the inflammatory effects of talcum powder have been demonstrated in humans, animals, and cells in culture. This study which clearly demonstrates malignant transformation of normal ovarian cells in culture adds to the strong evidence of a causal relationship between the genital use of talcum powder and ovarian cancer.

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311	Amy Harper: acquisition of data, study conception and design, critical revision
312	Xin Wang: acquisition of data
313	Rong Fan: acquisition of data
314	Nicole M. Fletcher: interpretation of data, study conception and design, critical revision
315	Robert T. Morris: interpretation of data, critical revision
316	Ghassan M. Saed: acquisition of data, study conception and design, drafting of
317	manuscript, critical revision
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343	VE	ICI	G1	16	62

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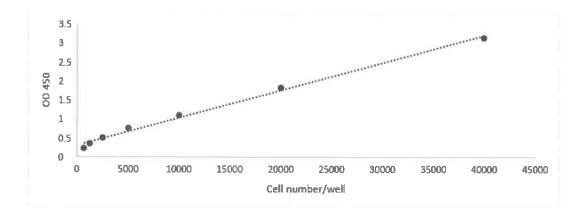
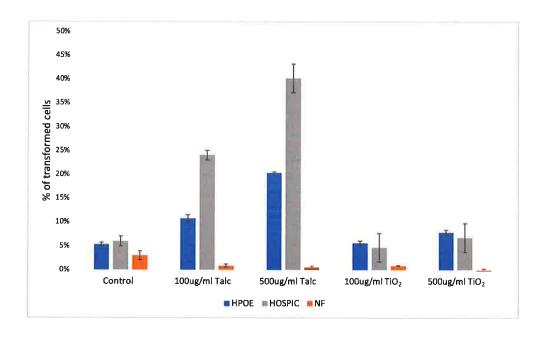


Figure 1: Human primary normal ovarian epithelial cell-dose curve. The cell dose curve was established as described in methods using a serial dilution of cells.



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Figure 2: Equal numbers (30K) of human primary normal ovarian epithelial cells (HPOE), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution. Control: cells (30K) with media only.

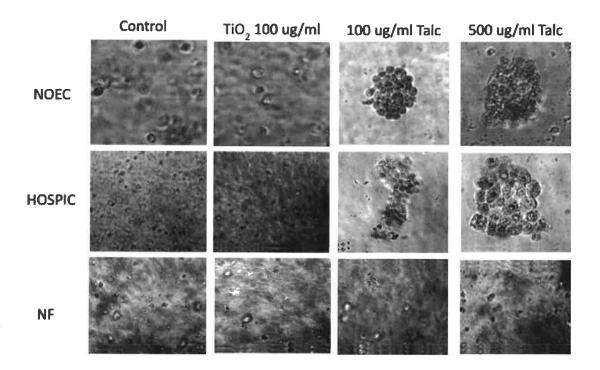
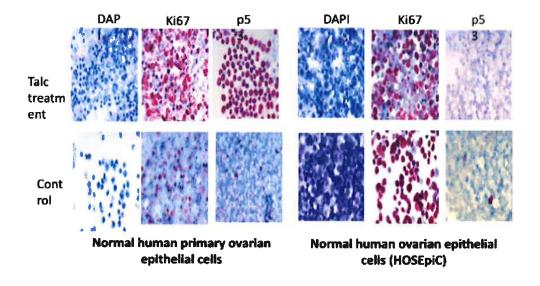


Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.



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Figure 4: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides were reviewed by two pathologists. Diffuse nuclear staining or complete negative staining with p53 is considered a positive reaction indicating mutated p53 status were observed in cells treated with talcum powder. Focal nuclear staining is consistent with wild type p53 and considered negative was observed in untreated cells (control). An increase in the proliferation index (Ki67) was observes in talcum powder treated cells versus controls.

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#### Highlights

- Exposure to talcum powder induces malignant transformation in ovarian epithelial cells
- Exposure to talcum powder did not induce malignant transformation in primary normal fibroblasts
- Mutant p53 type and increased expression of Ki67 were detected in ovarian cells when exposed to talcum powder



#### **Instructions**

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

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This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check

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This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

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This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

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1. Given Name (First Name) Ghassan	2. Surname (Last Name Saed	)	3. Date 04-January-2021	
4. Are you the corresponding author?	Yes No			
5. Manuscript Title Talcum powder induces malignant trans	formation in normal h	uman primary c	ovarian epith	nelial cells
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Name of Entity	Grant?	Personal Fees?	Non-Financial	Other?	Comments
Beasly Allen Law Firm		V		V	Served as a paid consultant and expert witness for the plaintiffs in the talcum powder litigation
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Intellectual Proper	ty Pate	ents & Cop	pyrights		
Do you have any patents, whether plant	ned, pendi	ing or issue	ed, broadly releva	nt to the	work? Yes V No
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Section 6. Disclosure Stateme	ent	57 1/1		11.0	
Based on the above disclosures, this form below.		omatically <u>c</u>	generate a disclos	sure state	ement, which will appear in the box
Dr. Saed reports personal fees from Beas Beasly Allen Law Firm, outside the subn	sly Allen La nitted wor	aw Firm, d k;.	uring the conduc	t of the s	tudy; personal fees and other from

## **Evaluation and Feedback**



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From: PLOS ONE em@editorialmanager.com

Subject: PLOS ONE Decision: PONE-D-20-29874 - [EMID:d5132363bdcb6a7c]

Date: October 28, 2020 at 9:17 AM

To: Ghassan M Saed gsaed@med.wayne.edu



## [EXTERNAL]

PONF-D-20-29874

Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells PLOS ONE

Dear Dr. Saed,

Thank you for submitting your manuscript to PLOS ONE. After careful consideration, we have decided that your manuscript does not meet our criteria for publication and must therefore be rejected.

Specifically:

Both reviewers have raised serious concerns about the experimental design, analyses and interpretation of the findings.

I am sorry that we cannot be more positive on this occasion, but hope that you appreciate the reasons for this decision.

Yours sincerely,

Salik Hussain, D.V.M, M.S., Ph.D., Academic Editor PLOS ONE

[Note: HTML markup is below. Please do not edit.]

Reviewers' comments:

Reviewer's Responses to Questions

#### Comments to the Author

1. Is the manuscript technically sound, and do the data support the conclusions?

The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

Reviewer #1: No

Reviewer #2: No

2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: No

Reviewer #2: No

3. Have the authors made all data underlying the findings in their manuscript fully available?

The PLOS Data policy requires authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception (please refer to the Data Availability Statement in the manuscript PDF file). The data should be provided as part of the manuscript or its supporting information, or deposited to a public repository. For example, in addition to summary statistics, the data points behind means, medians and variance measures should be available. If there are restrictions on publicly sharing data-e.g. participant privacy or use of data from a third party-those must be specified.

Reviewer #1: Yes

Reviewer #2: Yes

4. Is the manuscript presented in an intelligible fashion and written in standard English?

PLOS ONE does not copyedit accepted manuscripts, so the language in submitted articles must be clear, correct, and unambiguous. Any typographical or grammatical errors should be corrected at revision, so please note any specific errors

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Reviewer #1: Yes

Reviewer #2: No

#### 5. Review Comments to the Author

Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)

#### Reviewer #1: General Comments

The manuscript by Harper et al. entitled 'Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells' describes an in vitro study assessing talc powder ability to induce malignant transformation in human ovarian cell lines. The authors conducted a 72 hour exposure of talc powder or titanium dioxide to separate cultures of primary ovarian epithelial, ovarian epithelial, or primary peritoneal fibroblast cells. This was followed by plating exposed cells into agar-based cell transformation assay based on formazan dye absorbance measurement. The authors report a dose-dependent increase in transformed ovarian epithelial cells for only talc powder, and conclude that talc powder exposure causes malignant transformation in these cells. Although this study's subject is timely and warranted based on the current literature, several critical fatal flaws in the study included no description of talc powder and TiO2 characteristics, seeding density in the soft agar assay, and use of a single in vitro soft agar assay to claim malignant transformation. Critiques and suggestions for improvement are given as follows:

- 1) No hypothesis was stated in either the Abstract or the Introduction section. Please provide.
- 2) It was not clear or explained how an acute 72 hour exposure to talc powder leads to cell transformation. This suggests that a single application of talc is extremely potent and carries high risk for cell transformation. Since talc powder is widely used, why aren't cancer rates much, much higher? For most carcinogens, repeated exposures over long periods of time drive carcinogenesis. Without adequate discussion or further data to support this claim, this finding is highly questionable.

  3) In the abstract the authors state 'This finding represents a direct causation mechanism of talcum powder exposure.' Colony formation does not show molecular mechanism of action. At best, it describes the end product of a mechanism and a mode of action. Please revise.
- 4) Based on the minimal amount of data provided in this manuscript, the authors' conclusions suggesting acute exposure of talc powder to ovary epithelial cells is associated with ovarian cancer are outrageous and not supported by the manuscript's data.
- 5) The authors state their objective was 'to determine whether exposure to talcum powder will induce malignant transformation.' The in vitro method used did not address this objective. Soft agar colony formation alone in an in vitro test system is not enough data to claim malignant transformation. At best, the authors should claim 'cell transformation'. Malignant means cancer, while neoplasm refers cells that show tumor-like properties. To show 'neoplastic transformation', authors would need to conduct a more diverse battery of tests to show that these 'transformed' cells possess a tumor or cancer cell phenotype (i.e. cancer hallmarks), as outlined by Hannahan and Weinberg. Furthermore, the authors would need to show that the tested talc powder possesses a majority of the key characteristics that define a carcinogen (i.e. Smith et al. 2016), which is the set of criteria that IARC now uses in its carcinogen determinations. Some of these key characteristics are discussed, but no data is provided for this specific talc powder. Lastly, to clearly show that these cells were malignant, an in vivo experiment such as subcutaneous injection of these transformed cells into an immune compromised mouse model, would need to be conducted showing tumor growth and ability to invade neighboring tissue. All claims for 'malignant transformation' should be changed to 'cell transformation.'

  6) Page 3, Lines 19-20. The statement 'Previous in vitro studies have demonstrated a biologic effect when cells in culture
- are exposed to talc' is very generalized and vague. Please give specifics on what was previously reported in the literature. 7) The authors tested a lot of Johnson and Johnson talc powder that was not from a previously identified lot that contained asbestos fibers. Since the authors refer to asbestos fibers in talc powder possibly causing ovarian cancer and asbestos fibers are a known carcinogen, it is surprising that no particle characterization effort was performed. No polarized light or electron microscopy of talc particles was performed to investigate potential existence of fibers in the talc powder samples. In addition, what type of TiO2 was tested? Where was it acquired from? Most particle-based toxicity studies are now recommended to perform minimal particle characterization (size, shape, density, elemental analysis, surface area, surface reactivity, etc) as part of a published study since these factors contribute to toxicological response. Lastly, since this is a particle exposure conducted on a submerged in vitro culture, an estimate of deposited or internal dose should have been performed. The effective density of particles determined by particle characteristics, medium type, protein corona, etc can influence particle kinetics and cause differences in the deposited dose that the cells come into contact with.
- 8) The authors supply no justification for their chosen dose range. Is this a range that is expected in ovarian tissue with topical external application of talc powder? At what dose does talc become acutely toxic to ovarian cells? Please provide

9) The method for particle exposure to ovarian cells is not adequate. What was the seeding density in each well and what sylve method for particle exposure to ovarian cells is not adequate. What was the seeding density in each well and what size were used? What size were the talc powder particles and TiO2 particles in cell culture medium during exposure? How were exposed cells handled and transferred to the colony formation assay? Were the cells washed to remove any residual particle prior to agar plating? Figure 2 shows 'control' cells and 'negative control' cells? It was never clearly described what the difference between these treatment groups were. Please provide. Page 5 states that the negative control was a blank. If so, how can you have a positive percent transformed cell response for this treatment? 10) Page 5. The authors did not clearly state what the final concentration of exposed ovarian cells were in each well for the colony formation assay. This is critical since agar-based colony formation assays are susceptible to starting cell density. The authors state 'test cells were all made as 30 thousand cells/well' in the paragraph describing the standard curve method and again in Figure 2. Figure 3 left hand panels show a high density of cells in agar. Was this the colony transformation assay seeding density in each 96 well plate? If 30,000, this is a VERY large number since many previous publications using similar soft agar and agarose assays typically use ~1,000 cells per cm2. The methods described here suggest a 90,000 cells per cm2 seeding density. A low seeding density will test an individual cell's ability to survive nonattachment to substrate or other cells and grow into a suspended colony. I would guess that 30,000 cells were highly confluent in a 96-well plate and able to rely on each other for survival. These authors previously reported that talcexposed ovarian cells showed increased oxidative stress, proliferation, and some resistance to apoptosis. Near confluent, stressed epithelial cells can clump together in culture, but this is more of a stress response and not an indication of cell transformation associated with tumorigenesis. Other endpoints showing a transformed phenotype is needed. Lastly, transformed formazan dye is the endpoint and not a colony count. Hence high seeding density resulting in surviving cells not in a true colony is possible, and was not clear from the presented data. Since this the only data set supplied in this study, the transformation conclusion based on the results from unclear methodology is highly worrisome. 11) Based on the experimental design and presented data, a student t-test is an inappropriate test for this data set. A two or three-way analysis of variance (ANOVA) with dose, particle, and cell type would be a more appropriate statistical analysis. Did the authors test the assumptions to run a parametric statistical test? In addition, it is not clear what 'percent of transformed cells' refers to in Figure 2? What treatment was used as a control to determine xero%? It was not clearly described how percent of transformed cells was calculated? Please address.

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12) Page 3. The authors state 'several animal studies have reported that talcum powder causes inflammation'. The cited studies are not animal studies; they are human patient studies. Furthermore, the Wong et al. is a human patient study that concluded there was no link between talc use and ovarian cancer. In addition, the next sentence refers to in vitro studies and refers to large scale reviews. If animal and in vitro studies with specific effects need to be cited, please cite this primary literature and describe with enough detail to support the background argument.

- 1) Minor grammar errors were found throughout the manuscript. Please carefully review the entire manuscript.
- 2) Ug/ml is used instead of µg/ml. Please address all occurrences.
- 3) Reference #34 is the same as #3. Please omit. The first statement of the first full paragraph on Page 9 will need another source.

Reviewer #2: In this submission, Ghassan et al. aim to convince the reader that exposure of ovarian cells (both primary and non-primary) to talc powder leads to malignant transformations. However, this paper is written in such a manner, that the science cannot be trusted.

#### 1. Comments on abstract:

- a. In the second line, there should be a comma after the word "Here". It should read "Here, we determined...'
- b. In the literature, cells are more commonly described with the word "normal" being used first. Consider saying "normal human ovarian cell", "normal human primary ovarian cell" etc.
- c. Sixth line down, when mentioning doses, suggest re-wording to say "...were treated with either 100 or 500 ug/ml...."
  d. Last paragraph of abstract, second sentence. Suggested re-wording "These findings represent...."
- e. I would caution against using the word genital, as the reader would assume that would also include peritoneal fibroblast, but no transformations were observed in this cell line. Or perhaps, specify again that this was specific to the ovarian cells, but not fibroblasts.

#### 2. Comments on Introduction

- a. Second sentence needs re-wording. This reads better "...presents with various histopathologies, molecular biologies, and clinical outcomes, and is therefore considered....
- b. Should there be a word before screening methods? Last sentence of the first paragraph in the introduction. As written, it sounds like there is a lack of screening methods. If this is what the authors are trying to say, then it is fine. c. Consider the word "Eventual" before "development of chemoresistance" d. "The pathogenesis of EOC remains elusive...."

- e. Second paragraph, second sentence that begins with "epithelial ovarian cancer cells..." Are the authors referring to in vitro studies, in vivo studies, human studies? Please be more specific. The same goes for the sentence after that, please describe this in a more specific manner.
- f. Third paragraph of introduction, the word "Have" needs to be inserted after "meta-analyses".
- g. The first two sentences of the third paragraph of the introduction essentially say the same thing, consider re-writing this.

  h. In the animal studies described in the introduction, do the authors mean through dermal exposure? In general, the introduction lacks a lot of important specifics that would improve the quality of this submission.
- i. The sentence that starts with "previous in vitro studies" does not mention the cell type or anything important information that the readers needs.
- j. The last paragraph of the introduction is actually the most important, but the authors have written it in such a way that leaves more questions than answers. First off, silica and asbestos toxicity should get more attention in the introduction.

Second, why are the authors telling us about aspestos fibers in the lung? I his paper is about the genital use of taic powder, so please focus on that, or at least say that it could possibly initiate a similar inflammatory response in ovarian and fibroblast cells. However, the mechanism of cell toxicities in the lung are going to be vastly different that those observed in ovarian and fibroblast cells, so this last paragraph needs to be refined considerably.

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#### 3. Materials and Methods

- a. This section needs major revisions. Please make sub-categories within this section. For example, the primary human ovarian cells need their own sub-section, with a more detailed explanation of culture methods. Same goes for all cell lines, followed by a separate section for materials used (i.e. talc powder), and each method also needs its own sub-section (Cell Transformation Assay, WST etc..)
- b. Primary cells, in particular, require extra care and effort. The authors, for all cell lines, have just glazed over culture methods. What was the temperature and humidity and %CO2 in which these cells were kept? What extra steps were taken with the primary cells?
- c. Why was titanium dioxide chosen as a control? Reader needs to know this
- d. The lack of detail in this section makes this paper extremely difficult to follow. Please see all questions below General
- i. How were the doses of 100 ug/ml and 500 ug/ml chosen?
- ii. Where is the explanation that these doses are even relevant for the exposure model?
- iii. Where was the titanium dioxide obtained from?
- iv. Were the "suspensions" of talcum baby powder or titanium dioxide prepared freshly every time, or were they stored (either at room temp or in fridge)?
- v. What were the sonicator settings? What brand sonicator?
- vi. A much more detailed explanation of how cells were maintained and cultured/sub-cultured is needed.

#### Cell-transformation Assay

- vii. The authors fail to tell the reader what density of cells were used for these experiments. 24 wells? 96 wells? All relevant information is missing.
- viii. Was the medium changed or cells transferred to new plates prior to exposures?
- ix. "This assay was chosen because it is more stable, faster..."Please remove this poorly written sentence. Considered rewording "Compared to the (Traditional?) soft agar assay, this cell transformation assay was chosen because..." Alternately, there is no need to mention a comparison to the soft-agar assay because up until this point, it has not been on the reader's radar
- x. How were the results of the colorimetric cell transformation assay quantified? The authors make no mention of plate reader.
- xi. Did the authors leave the talcum powder suspensions or titanium dioxide suspensions in the cell culture wells for 72 hours straight? Or, was the medium replaced and refreshed? This needs to be made very clear
- xii. For controls, was cell viability measured over the course of 72 hour exposures to ensure that just sitting for 3 days with/without medium change (hard to tell, no information is given to reader) did not have an effect on viability?

#### WST Assay

- a. Authors says a cell-dose curve was established in manufacturer's protocol. What manufacturer are they referring to?
- b. Why is there so much detail in the WST assay, but not elsewhere?
- c. Where was WST obtained from?
- d. Was the WST assay done only after the 72 hour exposure? I would think that leaving the cells for 72 hours, the authors would check the viability of control cells every 24 hours to ensure that all decreases in viability were from exposures, and not just sitting (authors do not tell us cell culture conditions) for 72 hours.
- e. Authors do not tell readers what WST assay is for
- f. This entire description needs to be re-done g. Did the authors look at supernatants? How does that work, is the powder fully dissolved at this point or is it still a suspension? If powder is present in the supernatants tested, that will have an effect on colorimetric assays
- h. Were supernatants read right away? Stored for future use?
- i. WST-1 is normally used for viability, it is unclear how this played a role in identifying malignancies?

#### Agarose and WST

a. I have no idea what I am reading here. First the authors talk about an agarose assay, then WST, and now they are combined? This is very poorly explained.

The authors mention malignant transformations observed in cells, but where was the methodology for this? I did not see any carcinogenic assays

I did not see any methodology for detecting malignant transformations

If the premise of this paper is that talc paper causes cancer, it would make sense that the authors of this paper not only use talc powder, but perhaps try to get the individual components of the talc powder, so they can correctly identify what it is in the talc powder that is cancerous.

#### RESULTS

If the authors do not tell the reader which one of their methods was used to detect cell malignancies, then how is the reader supposed to just understand this results section?

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HOW exactly were transformed cens quantified? Osing the 14001-1 assay? Osing agaiose? 1401 is traditionally used for viability.

How were malignant colonies counted?

How do the authors have images of cells in their data, and make no mention of this in their methodology? Colorimetric assays were mentioned, and reading of absorbance, there was no mention of microscopy work/histology.

The problems with this submission are too numerous to count, and the science, methodology, and data cannot be trusted.

Discussion section cannot be trusted based on all of the numerous issues listed above.

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Reviewer #2: No

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Please consider our manuscript "Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells" for publication in your journal. We have previously published that Talcum powder induces an inflammatory/oxidative stress profile in normal epithelial ovarian cells similar to that seen in ovarian cancer cells. Here we are excited to demonstrate that exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding is intriguing, and work is currently ongoing in our laboratory to understand the mechanism.

Dr. Ghassan M. Saed Associate Professor of Gynecologic Oncology Director of Ovarian Cancer Biology Research Departments of Obstetrics and Gynecology and Oncology Member of Tumor Biology and Microenvironment Program **Karmanos Cancer Institute** Wayne State University School of Medicine Detroit, MI 48202 (313) 577-5433 Office (313) 577-1302 Lab (313) 577-4633 Fax.

## **PLOS ONE**

# Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells --Manuscript Draft--

Manuscript Number:					
Article Type:	Research Article				
Full Title:	Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells				
Short Title:	Talcum powder induces malignant transformation in ovarian cells				
Corresponding Author:	Ghassan M Saed, Ph.D. Wayne State University School of Medicine Detroit, Michigan UNITED STATES				
Keywords:	talc; talcum powder; titanium dioxide; epithelial ovarian cancer; transformation; primary cells; cell proliferation; colonies				
Abstract:	Several epidemiological and molecular studies have linked perineal use of talcum powder to increased risk of ovarian cancer. Here we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells. Human primary normal ovarian epithelial cells (Cell Biologics), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories), and human primary peritoneal fibroblasts were treated with 100 and 500 ug/ml of talcum powder or titanium dioxide (TiO2) as a control for 72 hours before assessment with a cell transformation assay. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in both ovarian cell lines. No colonies formed in the untreated ovarian cells or control ovarian cells at either dose. Interestingly, there were no colonies formed in talc treated normal peritoneal fibroblasts. Treatment with talc increased number of transformed ovarian cells by 11% and 20% in the 100 and 500 ug/ml doses, respectively (p<0.05). Likewise, but to a greater extent, treatment with talcum powder increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (p<0.05). There were no detectible transformed cells when treated with TiO2.  Exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding represents a direct causation mechanism of talcum powder exposure specific to normal ovarian cells and further supports previous studies of the association of genital use of talcum powder and increased risk of ovarian cancer.				
Order of Authors:	Ghassan M Saed, Ph.D.				
	Amy K. Harper				
	Xin Wang				
	Rong Fan				
	Nicole M. Fletcher				
	Robert T. Morris				
Opposed Reviewers:					
Additional Information:					
Question	Response				
Financial Disclosure  Enter a financial disclosure statement that describes the sources of funding for the	A portion of Dr. Saed's time conducting this research was paid for by the lawyers representing plaintiffs in the talcum powder litigation. Dr. Saed received no financial support for the authorship or publication of this article				

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Dr. Saed has served as a paid consultant and expert witness for the plaintiffs in the talcum powder litigation. The remaining authors have no potential conflicts of interest to report.

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Cover Letter

Dear sir/madam,

Please consider our manuscript "Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells" for publication in your journal. We have previously published that Talcum powder induces an inflammatory/oxidative stress profile in normal epithelial ovarian cells similar to that seen in ovarian cancer cells. Here we are excited to demonstrate that exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding is intriguing, and work is currently ongoing in our laboratory to understand the mechanism.

Dr. Ghassan M. Saed
Associate Professor of Gynecologic Oncology
Director of Ovarian Cancer Biology Research
Departments of Obstetrics and Gynecology and Oncology
Member of Tumor Biology and Microenvironment Program
Karmanos Cancer Institute
Wayne State University School of Medicine
Detroit, MI 48202
(313) 577-5433 Office
(313) 577-1302 Lab
(313) 577-4633 Fax

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# Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells

Amy K. Harper, M.D.<sup>1,2</sup>, Xin Wang, M.S.<sup>1</sup>, Rong Fan, M.S.<sup>1</sup>, Nicole M. Fletcher, Ph.D.<sup>1</sup>, Robert T. Morris, M.D.<sup>2</sup>, \*Ghassan M. Saed, Ph.D.<sup>1,2</sup>

<sup>1</sup> Department of Obstetrics and Gynecology, Wayne State University School of Medicine and <sup>2</sup> Department of Gynecologic Oncology, Karmanos Cancer Institute, 4100 John R,

Detroit, MI, 48201

aharper4@med.wayne.edu xin.wang7@wayne.edu florrie1522@gmail.com nfletche@med.wayne.edu rmorris@med.wayne.edu

Key words: talc, talcum powder, titanium dioxide, epithelial ovarian cancer, transformation, primary cells, cell proliferation, colonies

#### \*Corresponding Author:

Ghassan M. Saed, Ph.D.

Associate Professor of Gynecologic Oncology

Director of Ovarian Cancer Biology Research

Departments of Obstetrics and Gynecology and Oncology

Member of Tumor Biology and Microenvironment Program

Karmanos Cancer Institute

Wayne State University School of Medicine

Detroit, MI 48201

(313) 577-5433 Office phone, (313) 577-8544 Office fax, (313) 577-1302 Lab phone gsaed@med.wayne.edu

#### **Abstract**

Several epidemiological and molecular studies have linked perineal use of talcum powder to increased risk of ovarian cancer. Here we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells.

Human primary normal ovarian epithelial cells (Cell Biologics), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories), and human primary peritoneal fibroblasts were treated with 100 and 500 ug/ml of talcum powder or titanium dioxide (TiO<sub>2</sub>) as a control for 72 hours before assessment with a cell transformation assay.

Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in both ovarian cell lines. No colonies formed in the untreated ovarian cells or control ovarian cells at either dose. Interestingly, there were no colonies formed in talc treated normal peritoneal fibroblasts. Treatment with talc increased number of transformed ovarian cells by 11% and 20% in the 100 and 500 ug/ml doses, respectively (p<0.05). Likewise, but to a greater extent, treatment with talcum powder increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (p<0.05). There were no detectible transformed cells when treated with TiO<sub>2</sub>.

Exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding represents a direct causation mechanism of talcum powder exposure specific to normal ovarian cells and further supports previous studies of the association of genital use of talcum powder and increased risk of ovarian cancer.

#### Introduction

Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States (1). Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome is thus considered a heterogeneous disease (1, 2). The prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage (2). This is largely due to the lack of early warning symptoms, screening methods, and the development of chemoresistance (1, 2).

The pathogenesis of EOC is still being elucidated but has been strongly associated with oxidative stress and inflammation (3) (4). Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that has been demonstrated to be further enhanced in chemoresistant EOC cells (5),(3). Attenuation of the pro-oxidant state with antioxidants/scavengers has been shown to selectively induce apoptosis in EOC cells indicating a potential therapeutic value (5),(3).

The association between genital use of talcum powder and risk of EOC have been previously described (3, 6, 7). Several Meta-analyses demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder (8-10). In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress in animals (11) (12-14). Previous *in vitro* studies have demonstrated a biologic effect when cells in culture are exposed to talc (15-18). In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer (19).

On October 18, 2019, Johnson & Johnson recalled Johnson's Baby Powder lot #22318RB following FDA's finding that the product contained chrysotile asbestos. Talc

and asbestos are both silicate minerals; the carcinogenic effects of asbestos have been extensively studied and documented in the medical literature (3, 6). Asbestos fibers in the lung initiate an inflammatory and scarring process, and it has been proposed that ground talc, as a foreign body, might initiate a similar inflammatory response (3). Here we sought to determine whether exposure to talcum powder will induce malignant transformation in human primary normal ovarian epithelial cells and human primary normal peritoneal fibroblasts.

#### **Material and Methods**

Human primary normal ovarian epithelial cells (Cell Biologics, Chicago, IL), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories, Inc, Carlsbad, California), and human primary normal peritoneal fibroblasts were isolated and cultured as we have previously described (20, 21). The fibroblast cell line has been extensively characterized in previous studies and has been shown to be pure and solely fibroblast cells (20, 21). Human normal ovarian epithelial cells and HOSEpiC cells were grown in complete human epithelial cell medium and conditions following their manufacturer's protocols. Human normal primary peritoneal fibroblasts were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA). All media was supplemented with 10 % fetal bovine serum (FBS, Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific, Waltham, MA), per their manufacturer specifications.

Talcum baby powder (Johnson & Johnson, #30027477, Lot#13717RA) or Titanium dioxide (TiO<sub>2</sub>) were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each. Human primary normal ovarian epithelial cells and human primary

peritoneal fibroblasts were treated in duplicate with 100 and 500 u g/ml of talcum powder or TiO<sub>2</sub> as a control for 72 hours before assessment with cell transformation assay (colorimetric), according to the manufacturer protocol (Abcam-235698, Cambridge, MA). The experiments were repeated 3 times. This assay was chosen because it is more stable, faster and more sensitive than the Soft-Agar Assay.

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A Cell-dose curve was established as described in the manufacturer's protocol. Briefly, we used cells (5.34 x 10<sup>5</sup> cells/ml) were suspended in 1X DMEM/10% FBS medium. Cells were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was performed using an 8 channel multi pipette by adding 150 μl of media to each well of a 96 well microplate. A 150 μl aliquot of the 5.34 x10<sup>5</sup> cells/ml (80 x10<sup>3</sup> cells) was added to the wells of the first duplicate row. A 150 μl aliquot from the first duplicate row was removed and added to the next well and mixed. The process was repeated until the seven serial dilutions were obtained. The final well was for negative control (Blank) with media only and no cells. Test cells were all made as 30 x10<sup>3</sup> cells/well. A 35 μl aliquot of 1X DMEM/10% FBS and 15 μl of WST working solution were then added into each well and incubate at 37° C for 4 hours. The absorbance was measured by a microtiter plate reader at 450 nm (Figure 1).

Agarose and WST working solutions were prepared as described in the kit information sheet. The base agarose mix was added into the required wells and kept for 15 minutes at 4°C to solidify the agarose. Stock solution was prepared by using a solution of cells (1 X 10<sup>6</sup> cells/mL in 1X DMEM/10% FBS medium). The agarose-cell mix was added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10

min at 37°C, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm.

Statistical Analysis: Data was analyzed with paired t-tests. Data are expressed, as means +/- SD. Significance was determined as P <0.05.

#### Results

Treatment with talcum powder significantly increased number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Likewise, but to a greater extent, treatment with talcum powder significantly increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Talcum powder had no detectable transformation effect on normal peritoneal fibroblasts (Figure 2). Anchorage-independent growth is a hallmark of cancer cells. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in both of the normal epithelial ovarian cell lines in a dose dependent manner (Figure 3). Interestingly, there were no colonies formed in talcum powder treated normal fibroblasts (Figure 3). There were no colonies formed in either untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with the control, TiO<sub>2</sub>.

#### **Discussion**

This is the first study to directly show that exposure to talcum powder induces malignant transformation in normal human ovarian epithelial cells. Interestingly, the ability of talcum powder exposure to induce transformation appears to be specific to ovarian cells as it did not induce transformation in human normal peritoneal fibroblasts. (Figure 3). The reason for this specific effect of talcum powder on the ovaries in still under investigation. Talc and asbestos particles have been detected in pelvic lymph nodes and other pelvic organs including the ovaries (3, 6, 22-24). Several studies have pointed toward the peristaltic pump feature of the uterus and fallopian tubes, which is known to enhance transport of sperm into the oviduct ipsilateral to the ovary bearing the dominant follicle (6, 7, 25).

The first epidemiologic study suggesting an increased risk of ovarian cancer with the genital use of talcum powder was published by Cramer et al. in 1982. These studies have shown the development of lung tumors in female, not male rats exposed to talc (26). Additional studies have shown some tumor-like morphological changes and macrophage activation as a result of talc exposure (26-28). However, it has been reported that inhalation of talc for a prolonged time by experimental animals did not induce cancer (28-30). Collectively, these findings seem to suggest that direct contact of talc with ovarian cells is required to enhance the process of cellular transformation.

The epidemiologic association of talcum powder use and risk of ovarian cancer has been established (31, 32). The risks for ovarian cancer from genital talcum powder use vary by histologic subtype, menopausal status at diagnosis, hormone therapy use, weight, and smoking, however, necessary information about the frequency and duration

of usage were lacking (31-33). Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer.

Studies of other sources and batches of talcum powder as well as with other cell types are needed for a more comprehensive evaluation of the effect. Additional animal studies could further support the extent the effect of talcum powder. However, the result of this study clearly supports the molecular and epidemiological data on talc powder use and ovarian cancer risk.

Oxidative stress and inflammation have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes in EOC tissues and cells as compared to normal cells indicating an enhanced redox state (34). This redox state is further enhanced in chemoresistant EOC cells as evidenced by a further increase in key pro-oxidant enzymes and a decrease in anti-oxidant levels, suggesting a shift towards a pro-oxidant state (34). Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian (34, 35).

Our laboratory was the first to confirm the cellular effect of talcum powder and provide a potential molecular mechanism. Talcum powder exposure induced molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer(3, 19). In all talc-treated cells, there was a significant dose-dependent increase in key prooxidants with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory balance involves the induction of specific mutations

in key oxidant and antioxidant enzymes that correlate with alterations in their activities.

The fact that these mutations happen to correspond to known SNPs of these enzymes

suggests a genetic predisposition to developing ovarian cancer with genital talcum

powder use.

We have previously reported that EOC cells manifest increased cell proliferation

and decreased apoptosis (34). Recently, we have shown that talcum powder enhances

cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly

in normal cells, suggesting talc is a stimulus to the development of the oncogenic

phenotype. Talcum powder exposure also resulted in a significant increase in

inflammation as determined by increased tumor marker CA-125. These findings are the

first to confirm the cellular effect of talcum powder and provide a molecular mechanism

to previous reports linking genital use to increased ovarian cancer risk.

**Declaration of Conflicting Interests:** 

Dr. Saed has served as a paid consultant and expert witness for the plaintiffs in the talcum

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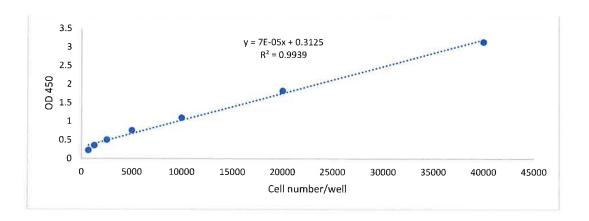
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Figure 1. Human primary normal ovarian epithelial cell-dose curve

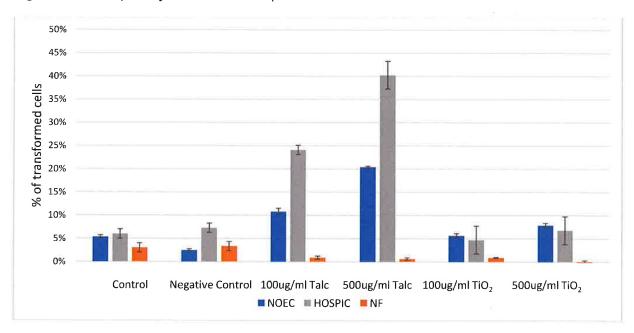


Figure 2. Equal numbers (30K) of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution.

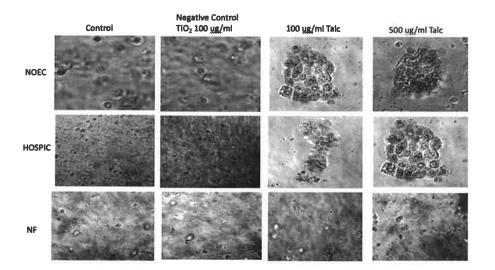


Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture.

From: Reproductive Sciences - Editorial Office em@editorialmanager.com

Subject: Your Submission RESC-D-20-00635 Date: August 27, 2020 at 12:24 AM

To: Ghassan Saed gsaed@med.wayne.edu

Dear Dr. Saed,

We have received the reports from our advisors on your manuscript RESC-D-20-00635 "Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells".

With regret, I must inform you that, based on the advice received, I have decided that your manuscript cannot be accepted for publication in Reproductive Sciences.

Below, please find the comments for your perusal. You are kindly requested to also check the website for possible reviewer attachment(s).

I would like to thank you very much for forwarding your manuscript to us for consideration and wish you every success in finding an alternative place of publication.

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Setsuko Chambers Associate Editor Reproductive Sciences

Comments to the author (if any):

- 1-The findings still would not establish that it is biologically plausible that talc causes ovarian cancer in living humans.
- 2- Injection talcum powder into the reproductive systems of laboratory animals to see if the same changes occur is highly recommended.
- 3-What are the Justification for the Talcum powder amount added to the cell cultures
- Are those amounts are in actual situations are present at the commercially used powder and how long the human needs to get these amounts in actual life .
- 4-Does the talcum powder used is pure or contains traces of asbestos
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## **Reproductive Sciences**

# Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells --Manuscript Draft--

Manuscript Number:	RESC-D-20-00635			
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Abstract:	Several epidemiological and molecular studies have linked perineal use of talcum powder to increased risk of ovarian cancer. Here we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells. Human primary normal ovarian epithelial cells (Cell Biologics), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories), and human primary peritoneal fibroblasts were treated with 100 and 500 u g/ml of talcum powder or titanium dioxide (TiO2) as a control for 72 hours before assessment with cell transformation assay. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in both ovarian cell lines. There were no colonies formed in untreated ovarian cells or control ovarian cells at either dose. Interestingly, there were no colonies formed in talc treated norma peritoneal fibroblasts. Treatment with talc increased number of transformed ovarian cells by 11% and 20% in the 100 and 500 ug/ml doses, respectively (p<0.05). Likewise, but to a greater extent, treatment with talcum powder increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powde doses, respectively (p<0.05). There were no detectible transformed cells when treated with TiO2. Exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding represents direct causation mechanism of talcum powder exposure specific to normal ovarian cells and further supports previous studies of the association of genital use of talcum powder and increased risk of ovarian cancer.			
Corresponding Author:	Ghassan Saed Wayne State University UNITED STATES			
Corresponding Author Secondary Information:				
Corresponding Author's Institution:	Wayne State University			
Corresponding Author's Secondary Institution:				
First Author:	Amy K. Harper, M.D.			
First Author Secondary Information:				
Order of Authors:	Amy K. Harper, M.D.			
	Xin Wang, M.S.			
	Rama Majed, B.S.			
	Rong Fan, M.S.			
	Nicole M. Fletcher, Ph.D.			
	Robert T. Morris, M.D.			
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Amy K. Harper, M.D.a, Xin Wang, M.S.a, Rama Majed, B.S.a, Rong Fan, M.S.a, Nicole M.

Fletcher, Ph.D.a, Robert T. Morris, M.D.b, Ghassan M. Saed, Ph.D.a,b

<sup>a</sup> Department of Obstetrics and Gynecology, Wayne State University School of Medicine and b Department of Gynecologic Oncology, Karmanos Cancer Institute, Detroit, MI

Corresponding Author:

Ghassan M. Saed, Ph.D.

Associate Professor of Gynecologic Oncology

Director of Ovarian Cancer Biology Research

Departments of Obstetrics and Gynecology and Oncology

Member of Tumor Biology and Microenvironment Program

Karmanos Cancer Institute

Wayne State University School of Medicine

Detroit, MI 48201

(313) 577-5433 Office phone

(313) 577-8544 Office fax

(313) 577-1302 Lab phone

gsaed@med.wayne.edu

#### **Abstract**

Several epidemiological and molecular studies have linked perineal use of talcum powder to increased risk of ovarian cancer. Here we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells. Human primary normal ovarian epithelial cells (Cell Biologics), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories), and human primary peritoneal fibroblasts were treated with 100 and 500 u g/ml of talcum powder or titanium dioxide (TiO<sub>2</sub>) as a control for 72 hours before assessment with cell transformation assay. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in both ovarian cell lines. There were no colonies formed in untreated ovarian cells or control ovarian cells at either dose. Interestingly, there were no colonies formed in talc treated normal peritoneal fibroblasts. Treatment with talc increased number of transformed ovarian cells by 11% and 20% in the 100 and 500 ug/ml doses. respectively (p<0.05). Likewise, but to a greater extent, treatment with talcum powder increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (p<0.05). There were no detectible transformed cells when treated with TiO2. Exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding represents a direct causation mechanism of talcum powder exposure specific to normal ovarian cells and further supports previous studies of the association of genital use of talcum powder and increased risk of ovarian cancer.

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Keywords: talc, talcum powder, titanium dioxide, epithelial ovarian cancer, transformation, primary cells, cell proliferation, colonies

#### Introduction

Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States [1]. Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome is thus considered a heterogeneous disease [1, 2]. The prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage [2]. This is largely due to the lack of early warning symptoms, screening methods, and the development of chemoresistance [1, 2]. The pathogenesis of EOC is still being elucidated but has been strongly associated with oxidative stress and inflammation.

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Epithelial ovarian cancer has been strongly associated with oxidative stress and inflammation [3] [4]. Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that is further enhanced in chemoresistant EOC cells [5],[3]. Attenuation of the prooxidant state with antioxidants/scavengers have been shown to selectively induce apoptosis in EOC cells indicating a potential therapeutic value [5],[3].

The association between genital use of talcum powder and risk of EOC have been previously described [3, 6, 7]. Several Meta-analyses demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder [8-10]. In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress in animals [11] [12-14]. Previous in vitro studies have demonstrated a biologic effect when cells in culture are exposed to talc [15-18]. In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer [19].

On October 18, 2019, Johnson & Johnson recalled Johnson's Baby Powder lot #22318RB following FDA's finding that the product contained chrysotile asbestos. Talc and asbestos are both silicate minerals; the carcinogenic effects of asbestos have been extensively studied and documented in the medical literature [3, 6]. Asbestos fibers in the lung initiate an inflammatory and scarring process, and it has been proposed that ground talc, as a foreign body, might initiate a similar inflammatory response [3]. Here we sought to determine whether exposure to talcum powder will induce malignant transformation in human primary normal ovarian epithelial cells and human primary normal peritoneal fibroblasts.

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#### **Material and Methods**

Human primary normal ovarian epithelial cells (Cell Biologics, Chicago, IL), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories, Inc. Carlsbad, California), and human primary normal peritoneal fibroblasts were isolated and cultured as we have previously described [20, 21] . The fibroblast cell line has been extensively characterized in previous studies and has been shown to be pure and solely fibroblast cells [20, 21]. Human normal ovarian epithelial cells and HOSEpiC cells were grown in complete human epithelial cell medium and conditions following their manufacturer's protocols. Human normal primary peritoneal fibroblasts were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA). All media was supplemented with 10 % fetal bovine serum (FBS, Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific), per their manufacturer specifications.

Talcum powder or Titanium dioxide (TiO<sub>2</sub>) were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each. Human primary normal ovarian epithelial cells and human primary peritoneal fibroblasts were treated in duplicate with 100 and 500 u g/ml of talcum powder or TiO<sub>2</sub> as a control for 72 hours before assessment with cell transformation assay (colorimetric), according to the manufacturer protocol (abcam 235698). The experiments were repeated 3 times. This assay was chosen because it is more stable, faster and more sensitive than the Soft-Agar Assay.

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Cell-dose curve was established as described in manufacturer protocol. Briefly, we used cells (5.34 x 105 cells/ml) in 1X DMEM/10% FBS medium and a control containing no cells. Cells were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was performed using an 8 channel multi pipette by adding 150 µl of media to each well of a 96 well microplate. One hundred fifty µl aliquot of 5.34 x105 cells/ml (80 x103 cells) was added to the wells of the first duplicate row. One hundred fifty µl aliquot from the first duplicate row was removed and added to the next well and mixed. The process was repeated until the seven serial dilutions were obtained. The final well was for negative control (Blank) with media only and no cells. Test cells were all made as 30 x10<sup>3</sup> cells/well. A 35 μl aliquot of 1X DMEM/10% FBS and 15 μl of WST working solution were then added into each well and incubate at 37° C for 4 hours. The absorbance was measured by a microtiter plate reader at 450 nm.

Agarose and WST working solutions were prepared as described in the kit information sheet. The base agarose mix was added into the required wells and kept for 15 minutes at 4°C to solidify the agarose. Stock solution was prepared by using a solution of cells (1 X 106 cells/mL in 1X DMEM/10% FBS medium). The agarose-cell mix was

added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10 min at 37°C, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm.

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Statistical Analysis: Data was analyzed with paired t-tests. Data are expressed, as means +/- SD. Significance was determined as P <0.05.

#### Results

Treatment with talcum powder significantly increased number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 3, p<0.05). Likewise, but to a greater extent, treatment with talcum powder significantly increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, p<0.05). Talcum powder has no detectable transformation effect on normal peritoneal fibroblasts (Figure Anchorage-independent growth is a hallmark of cancer cells. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in both of the normal epithelial ovarian cell lines in a dose dependent manner (Figure 3). There were no colonies formed in untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with TiO2. Interestingly, there were no colonies formed in talcum powder treated normal fibroblasts (Figure 3).

#### **Discussion**

This is the first study to directly show that exposure to talcum powder induces malignant transformation in normal human ovarian epithelial cells. Interestingly, the ability of talcum powder exposure to induce transformation appears to be specific to ovarian cells as it did not induce transformation in human normal peritoneal fibroblasts. (figure 3). The reason for this specific effect of talcum powder on the ovaries in still under investigation. Talc and asbestos particles have been detected in pelvic lymph nodes and other pelvic organs including ovaries [6, 3, 22-24]. Several studies have pointed toward the peristaltic pump feature of the uterus and fallopian tubes, which is known to enhance transport of sperm into the oviduct ipsilateral to the ovary bearing the dominant follicle [6, 7, 25].

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The epidemiologic association of talcum powder use and risk of ovarian cancer has been established [31, 32]. The risks for ovarian cancer from genital talcum powder use vary by histologic subtype, menopausal status at diagnosis, hormone therapy use, weight, and smoking however, necessary information about the frequency and duration

of usage were lacking [32, 31, 33]. Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer.

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Studies of other sources and batches of talcum powder as well as with other cell types are needed for a more comprehensive evaluation of the effect. Additional animal studies could further support the extent the effect of talcum powder. However, the result of this study clearly supports the molecular and epidemiological data on talc powder use and ovarian cancer risk.

Oxidative stress and inflammation have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes in EOC tissues and cells as compared to normal cells indicating an enhanced redox state [3]. This redox state is further enhanced in chemoresistant EOC cells as evidenced by a further increase in key pro-oxidant enzymes and a decrease in anti-oxidant levels, suggesting a shift towards a pro-oxidant state [3]. Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian [3, 34].

Our laboratory was the first to confirm the cellular effect of talcum powder and provide a potential molecular mechanism. Talcum powder exposure induced molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer[3, 19]. In all talc-treated cells, there was a significant dose-dependent increase in key prooxidant with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory balance involves the induction of specific mutations

in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes indicate a genetic predisposition to developing ovarian cancer with genital talcum powder use.

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We have previously reported that EOC cells manifest increased cell proliferations and decreased apoptosis [3]. Recently, we have shown that talcum powder enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype. Talcum powder exposure also resulted in a significant increase in inflammation as determined by increased tumor marker CA-125. These findings are the first to confirm the cellular effect of talcum powder and provide a molecular mechanism to previous reports linking genital use to increased ovarian cancer risk.

#### **Declaration of Conflicting Interests:**

Dr. Saed has served as a paid consultant and expert witness for the plaintiffs in the talcum powder litigation. The remaining authors have no potential conflicts of interest to report.

#### Funding:

A portion of Dr. Saed's time conducting this research was paid for by the lawyers representing plaintiffs in the talcum powder litigation. Dr. Saed received no financial support for the authorship or publication of this article.

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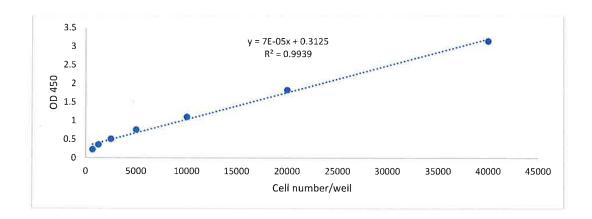


Figure 1. Human primary normal ovarian epithelial cell-dose curve

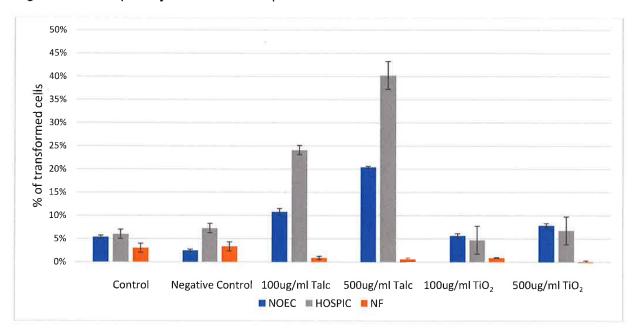


Figure 2. Equal numbers (30K) of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution.

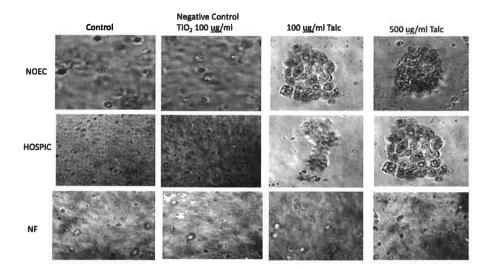


Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture.

ethical consents

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This is an observational study which utilizes commercially available cell lines. No ethical approval is required.

From: Dale Gibbons dale.gibbons@sgo.org & Subject: Abstract #15665 for SGO 2020 Annual Meeting

Date: January 31, 2020 at 8:31 AM

To: aharper4@med.wayne.edu, florrie1522@gmail.com, gb1258@wayne.edu, nicolefletcherking@gmail.com,

rmorris@med.wayne.edu, gsaed@med.wayne.edu

Cc: Kim, Kenneth kkim@uabmc.edu, Moore, Kathleen N. (OB/GYN - HSC) Kathleen-Moore@ouhsc.edu

Dear Drs. Harper, Fan, Majed, King, Morris, and Saed,

I am writing to you on behalf of SGO's 2020 Annual Meeting Program Committee Cochairs, Drs. Kathleen Moore and Kenneth Kim. Drs. Moore and Kim would like to know if you would like to update your disclosures for the attached abstract. Please let me know as soon as possible so we can accurately report the disclosures in the printed program book.

You may email any updates directly to me.

Please contact me if you have any questions.

Thank you,

Dale

Dale Gibbons, CAE DIRECTOR OF PROFESSIONAL EDUCATION | SOCIETY OF GYNECOLOGIC **ONCOLOGY | FOUNDATION FOR WOMEN'S CANCER** 

dale.gibbons@sgo.org | sgo.org | foundationforwomenscancer.org Direct: 312-676-3918 | Main Office: 312-235-4060, ext. 1258 | Fax: 312-235-4059 230 W. Monroe St., Ste. 710, Chicago, IL 60606-4703 USA

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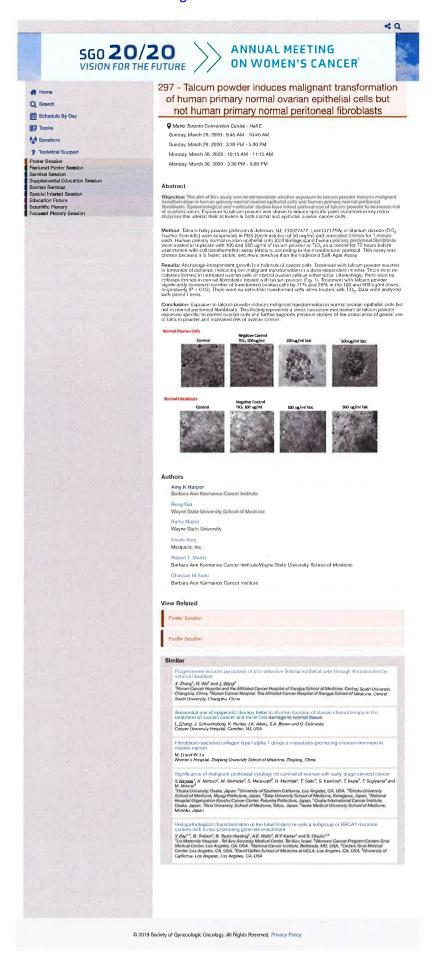




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15665 Talcum powder...ls.docx



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Subject: SGO Abstract

Date: September 9, 2019 at 11:13 AM



To: Amy Harper aharper4@med.wayne.edu, Nicole King nfletche@med.wayne.edu, Robert Morris rmorris@med.wayne.edu

I am submitting this abstract to SGO. Please edit and let me know if you have any changes!

- > Dr. Ghassan M. Saed
- > Associate Professor of Gynecologic Oncology
- > Director of Ovarian Cancer Biology Research
- > Departments of Obstetrics and Gynecology and Oncology
- > Member of Tumor Biology and Microenvironment Program
- > Karmanos Cancer Institute
- > Wayne State University School of Medicine
- > Detroit, MI 48202
- > (313) 577-5433 Office
- > (313) 577-1302 Lab
- > (313) 577-4633 Fax





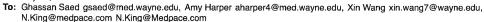
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From: Robert Morris rmorris@med.wayne.edu

Subject: Re: Manuscript

Date: July 20, 2020 at 12:38 PM



The manuscript looks great. A couple questions. Why was TiO2 selected as a control? Does this need to be explained in the manuscript? Are the concentrations used physiologically possible (especially in the ovary)?

#### Bob

From: Ghassan Saed <gsaed@med.wayne.edu>

Sent: Thursday, July 9, 2020 2:55 PM

To: Amy Harper <aharper4@med.wayne.edu>; Robert Morris <rmorris@med.wayne.edu>; Xin Wang <xin.wang7@wayne.edu>; N.King@medpace.com < N.King@Medpace.com>

Subject: Manuscript

Dear All,

The manuscript is ready for submission. Please let me know should you have any corrections or changes. Please use track changes.

### Best regards Ghassan

- > Dr. Ghassan M. Saed
- > Associate Professor of Gynecologic Oncology
- > Director of Ovarian Cancer Biology Research
- > Departments of Obstetrics and Gynecology and Oncology
- > Member of Tumor Biology and Microenvironment Program
- > Karmanos Cancer Institute
- > Wayne State University School of Medicine
- > Detroit, MI 48202
- > (313) 577-5433 Office
- > (313) 577-1302 Lab
- > (313) 577-4633 Fax

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

#### **BIOGRAPHICAL SKETCH**

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Amy Harper

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Fellow

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Cedarville University, Cedarville, OH	B.S.	05/2009	Biology
University of Toledo College of Medicine and Life Sciences, Toledo, OH	M.D.	06/2014	Medicine
MD Anderson Cancer Center, Felix Rutledge Fellowship, Houston, TX		11/2017	Gynecologic Oncology
The University of Maryland School of Medicine, Obstetrics and Gynecology Residency, Baltimore, MD	Residency	06/2018	Obstetrics and Gynecology
Wayne State University/Karmanos Cancer Institute, Gynecologic Oncology Fellowship, Detroit, MI			Gynecologic Oncology

#### A. Personal Statement

I am currently a fellow in the Gynecologic Oncology fellowship program at Wayne State University/Karmanos Cancer Institute in Detroit, Michigan, where I am completing my training in the clinical care of women with gynecologic malignancies and in the research of this challenging field of medicine. I have participated in several basic science and translational research projects throughout my undergraduate and graduate education, which has prepared me well for taking on more complex research endeavors in fellowship. Having completed a rigorous residency at the University of Maryland in obstetrics and gynecology and the Felix Rutledge Fellowship in gynecologic oncology at the MD Anderson Cancer Center. I am able to relate the data gathered at the laboratory bench to the patients we see in the clinic and on the wards.

As a research fellow, I am directly participating in the development of research strategies, acquisition and interpretation of data, and manuscript writing under the mentorship of the Principal Investigator, Dr. Ghassan Saed. With his guidance and expertise, we have been able to publish in a peer-reviewed journal and present several posters at the Society for Gynecologic Oncology Annual Meeting and the Society for Reproductive Investigation Annual Meeting. I am thrilled to continue to collaborate with Dr. Saed for this exciting and promising proposed investigation.

#### **Positions and Honors** В.

2008-2010 Research Assistant, Dept. of Physiology and Biophysics, Case Western Reserve University,

Cleveland, OH

2014-2018 Obstetrics and Gynecology Resident, University of Maryland School of Medicine, Baltimore,

MD 2018

Administrative Chief Ob/Gyn Resident, University of Maryland School of Medicine, Baltimore,

MD

Gynecologic Oncology Fellow, Wayne State University School of Medicine/Karmanos Cancer Institute,

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Detroit, MI

Honors:

2013 Gold Humanism Honor Society

2014 Alpha Omega Alpha Honor Medical Society

2017 Felix Rutledge Fellow, MD Anderson Cancer Center, Houston, TX

2018 Resident Research Award, Dept. of Obstetrics and Gynecology, University of Maryland School

of

Medicine, Baltimore, MD

#### C. **Contributions to Science**

1) Effects of alcohol consumption on liver metabolism: Identified the occurrence of a specific damage of hepatic G6Pase following acute alcohol administration. Highlighted the consequence of such damage for the ability of hepatic endoplasmic reticulum to sequester and mobilize its Ca2+ store. Identified that acute administration of low doses of EtOH is sufficient to dysregulate Ca2+ uptake and cycling within the hepatocyte by impairing the hydrolytic activity of G6Pase and the production of inorganic phosphate within the endoplasmic reticulum. This dysregulation occurs to a greater extent following the acute administration of a high dose of EtOH, as the alcohol not only inhibits the G6Pase activity to a larger extent but also impairs the operation of receptors and pumps within the cell membrane, further compromising Ca<sup>2+</sup> homeostasis within the hepatocyte. This dysregulation can have severe and far reaching implication for cell bioenergetics (i.e. mitochondrial uncoupling by Ca2+) and gene activation. Jacobs-Harper, A., Crumbly, A., Romani, A. Acute Effect of Ethanol on Hepatic Reticular G6Pase and Ca2+ Pool. Alcoholism: Clinical and Experimental Research. 37(s1): E40 - E51, January 2013.

2) Implications of EP4 receptor and class III-β tubulin expression on prognosis and treatment of uterine smooth muscle tumors: Class III-b tubulin is known to convey a resistant to taxanes in the treatment of several different cancers. As a mainstay of therapy for gynecologic malignancies, and specifically uterine smooth muscle tumors, this poses a significant hurdle for successful treatment. Receptor EP4 has been identified as an alternative target in a number of different cancers. I investigated the degree of expression of class III-b tubulin and EP4 receptor within human benign and malignant uterine smooth muscle tumors in a large group of human subjects and determined the effect on progression free survival and overall survival. Using commercially available human cell lines, I developed a model for uterine leiomyosarcoma and determined the cytotoxic effect of a proprietary, small molecule drug on this aggressive and devastating malignancy.

Harper, A., Reader, J., Legesse, T., Rao, G., Roque, D., Staats, P. EP4 receptor and class III-β tubulin expression in uterine smooth muscle tumors: Implications for prognosis and treatment. Gynecologic CancerSociety. 16th Biennial Meeting, Lisbon. Portugal, October 29-31, 2016.

3) Treatment modalities of uterine sarcoma: Uterine sarcomas are characterized by poor response to systemic chemotherapy and high recurrence rates. I investigated whether the use of cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (HIPEC) confers survival benefit in comparison with conventional treatment modalities in patients with recurrent US.

Díaz-Montes TP, El-Sharkawy F, Lynam S, Harper A, Sittig M, MacDonald R, Gushchin V, Sardi A. Efficacy of Hyperthermic Intraperitoneal Chemotherapy and Cytoreductive Surgery in the Treatment of Recurrent Uterine Sarcoma. Int J Gynecol Cancer. 2018;28:1130-37. doi: 10.1097/IGC.000000000001289.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

#### **BIOGRAPHICAL SKETCH**

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Nicole M. King (nee Fletcher)

eRA COMMONS USER NAME (credential, e.g., agency login): aj5932

POSITION TITLE: Postdoctoral Fellow

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY	
Wayne State University, Detroit, MI	B.S.	08/2006	Biological Sciences	
Wayne State University School of Medicine, Detroit, MI	Ph.D.	08/2013	Physiology, Concentration in Reproductive Science	
Wayne State University School of Medicine, Detroit, MI	Postdoctoral Training	08/2013-Pre sent	Cancer and infertility research	

#### A. **Personal Statement**

My research has focused on investigating the role of oxidative stress in the pathophysiology of gynecologic fibrotic disorders. Specifically, I have been involved in studying the effects of oxidative stress on the pathogenesis of ovarian cancer as well as in benign overgrowths, such as postoperative adhesions, fibroids, and endometriosis. Our laboratory has identified an important role for myeloperoxidase (MPO) and inducible nitric oxide synthase, key enzymes regulating oxidative stress, in the pathophysiology of ovarian cancer. This work has provided the foundation for this proposal. Specifically, we are the first to report MPO expression in epithelial ovarian cancer cells and tissues, but not in normal epithelial ovarian tissues. Much of my doctoral and postdoctoral work has focused on the role of both oxidants and antioxidants in ovarian cancer as compared to normal ovarian epithelial cells and chemoresistant ovarian cancer cells, and serves as preliminary data and support for this grant proposal. I have experience in performing all assays pertaining to this proposal as evident by the below highlighted studies (1-4). I will execute all experiments as well as writing abstracts, manuscripts, and grants pertaining to this work.

- 1. Saed GM, Ali-Fehmi R, Jiang ZL, Fletcher NM, Diamond MP, Abu-Soud HM, et al. Myeloperoxidase serves as a redox switch that regulates apoptosis in epithelial ovarian cancer. Gynecologic oncology. 2010;116:276-81. PMID: 19962178; PMCID: PMC2834266.
- 2. Belotte J, Fletcher NM, Awonuga AO, Alexis M, Abu-Soud HM, Saed MG, et al. The role of oxidative stress in the development of cisplatin resistance in epithelial ovarian cancer. Reproductive sciences. 2014;21:503-8. PMID: 24077440.
- 3. Saed GM, Diamond MP, Fletcher NM. Updates of the role of oxidative stress in the pathogenesis of ovarian cancer. Gynecol Oncol. 2017 June;145(3):595-602. Epub 2017 Feb 23. Review. PMID: 28237618.
- 4. Saed GM, Fletcher NM, Diamond MP, Morris RT, Gomez-Lopez N, Memaj I. Novel expression of CD11b in epithelial ovarian cancer: potential therapeutic target. Gynecologic Oncology. December 2017. In Press.

#### B. **Positions and Honors**

2002	Dean's list, Wayne State University, Detroit, MI
2002	Blue Cross Blue Shield Superior Academic Performance Scholarship - Winter
2003	Blue Cross Blue Shield Superior Academic Performance Scholarship - Fall
2007-2008	Research Assistant, Wayne State University School of Medicine, Detroit, MI
2008-2013	Interdisciplinary Biological Science Fellowship, Wayne State Univ., Detroit, MI
2008-2013	Graduate Research Assistant, Wayne State University School of Medicine, Detroit, MI
2012-present	Director of Laboratory Operations, DS Biotech, LLC, Detroit, MI
2013-present	Postdoctoral Fellow, Wayne State University School of Medicine, Detroit, MI

#### C. Below are my significant contributions to science.

- 1) The role of oxidative stress in the pathogenesis of ovarian cancer: the discovery of myeloperoxidase expression in ovarian cancer. We have characterized epithelial ovarian cancer (EOC) cells and tissues to manifest a unique oxidative stress profile, which is further altered in chemoresistance (5). While MPO was previously recognized to be present only in hemopoietic cells, we were the first to report MPO expression in solid ovarian malignancies and EOC cells (6). We have also demonstrated a functional cross talk between MPO and inducible nitric oxide synthase (iNOS), a key oxidant enzyme, where MPO utilizes nitric oxide (NO) as a substrate and converts it to nitrosonium cation, which nitrosylates the caspase-3 active site, leading to inhibition of EOC cells apoptosis, a hallmark of cancer cells (6). Furthermore, reduction of enhanced oxidative stress-induced apoptosis of EOC cells (7). Additionally, we have defined a role for MPO in vitro under oxidative stress and in vivo in EOC cells to be associated with advanced stage as compared with early stage ovarian cancer (8). More importantly, we demonstrated a significant increase in the levels of MPO and free iron in serum and tissues obtained from early stage ovarian cancer as compared to late stages, as well as compared to benign gynecologic conditions or healthy controls. Collectively, these findings strongly support a role for serum MPO and free iron in the pathophysiology of ovarian cancer, which thereby qualifies it as biomarkers for early detection of ovarian cancer (8). Significantly, the innovative clinical observation is uniquely paired with a molecular biologic process, which provides a plausible mechanism for defining a specific role for these key oxidants enzymes in the pathophysiology of ovarian cancer.
- 5. Belotte J, Fletcher NM, Awonuga AO, Alexis M, Abu-Soud HM, Saed MG, et al. The Role of Oxidative Stress in the Development of Cisplatin Resistance in Epithelial Ovarian Cancer. Reproductive sciences. 2013. PMID: 24077440.
- 6. Saed GM, Ali-Fehmi R, Jiang ZL, Fletcher NM, Diamond MP, Abu-Soud HM, et al. Myeloperoxidase serves as a redox switch that regulates apoptosis in epithelial ovarian cancer. Gynecologic oncology, 2010;116:276-81. PMID: 19962178; PMCID: PMC2834266.
- 7. Saed GM, Fletcher NM, Jiang ZL, Abu-Soud HM, Diamond MP. Dichloroacetate induces apoptosis of epithelial ovarian cancer cells through a mechanism involving modulation of oxidative stress. Reproductive sciences. 2011;18:1253-61. PMID: 21701041.
- 8. Fletcher NM, Jiang Z, Ali-Fehmi R, Levin NK, Belotte J, Tainsky MA, et al. Myeloperoxidase and free iron levels: potential biomarkers for early detection and prognosis of ovarian cancer. Cancer Biomark. 2011;10:267-75. PMID: 22820082.
- 2) A Single Nucleotide Polymorphism in Catalase Is Strongly Associated with Ovarian Cancer Survival. We have clearly established that oxidative stress plays a major role in the pathogenesis of ovarian cancer, however the exact mechanisms remain to be clarified. There is an emerging consensus that most of the genetic component of ovarian cancer risk is due to genetic polymorphisms that confer low to moderate risk (9). Single nucleotide polymorphisms (SNPs) occur because of point mutations that are selectively maintained in

populations that are distributed throughout the human genome at an estimated overall frequency of at least one in every 1000 base pairs (9). Non-synonymous SNPs substitute encoded amino acids in proteins, and are more likely to alter the structure, function, and interaction of the protein (9). Recent evidence demonstrates an association between enzymatic activity altering single nucleotide polymorphisms (SNP) with human cancer susceptibility (10). Chemoresistant ovarian cancer cells manifested specific point mutations, which are associated with altered enzymatic activity, in key redox enzymes that are not detected in sensitive counterparts (9). Supplementation of an antioxidant was able to successfully sensitize ovarian cancer cells to chemotherapeutics. Thus, we have now demonstrated that chemotherapy induces specific point mutations in key redox enzymes, which contributes to the acquisition of chemoresistance in ovarian cancer cells, highlighting a potential novel mechanism (9). Identification of targets for chemoresistance with either biomarker and/or screening potential will have a significant impact for the treatment of this disease. We also sought to evaluate the association of SNPs in key oxidant and antioxidant enzymes with increased risk and survival in epithelial ovarian cancer. Individuals (n = 143) recruited were divided into controls, (n = 94): healthy volunteers, (n = 18), high-risk BRCA1/2 negative (n = 53), high-risk BRCA1/2 positive (n = 23) and ovarian cancer cases (n = 49). This study indicates a strong association with the catalase SNP and survival of ovarian cancer patients, and thus may serve as a prognosticator (10).

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- 9. Fletcher NM, Belotte J, Saed MG, Memaj I, Diamond MP, Morris RT, Saed GM. Specific point mutations in key redox enzymes are associated with chemoresistance in epithelial ovarian cancer. Free Radic Biol Med. 2017 Jan; 102:122-132. PMID: 27890641.
- 10. \*Belotte J, \*Fletcher NM, Saed MG, Abusamaan MS, Dyson G, Diamond MP, et al. A Single Nucleotide Polymorphism in Catalase Is Strongly Associated with Ovarian Cancer Survival. PloS one. 2015;10:e0135739. PMID: 26301412; PMCID: PMC4547699. \*Indicates co-authorship.
- 3) The discovery of the adhesion phenotype. An additional significant contribution is the discovery of the "adhesion pheontype." We have shown that fibroblast cultures established from fresh adhesion tissues manifest a unique phenotype that resembles myofibroblasts, which we have termed the "adhesion phenotype." Specifically, the adhesion phenotype is characterized by increased α-smooth muscle actin, increased transforming growth factors and cytokines and extracellular matrix production, decreased extracellular matrix turnover via effects on matrix-degrading enzymes and their inhibitors, as well as decreased apoptosis (11). Remarkably, hypoxia causes normal peritoneal fibroblasts to irreversibly acquire the adhesion phenotype (12). The generated superoxide  $(O_2^{\bullet -})$  has been previously demonstrated to be a key player in formation of the adhesion phenotype, and hypoxia induced an oxidative stress environment that favors the development of this phenotype (12). Adhesion fibroblasts manifest decreased superoxide dismutase (SOD3), caspase-3 activity. nitrate/nitrite (NO<sub>3</sub><sup>-/</sup> NO<sub>2</sub><sup>-</sup>), as well as increased NADPH oxidase, S-nitrosylation of caspase-3, and lipid peroxidation, as compared to normal peritoneal fibroblasts (11). Hypoxia simultaneously increases extracellular matrix production and decreases turnover via effects on matrix-degrading enzymes and their inhibitors leading to the manifestation of an adhesion phenotype. The mechanism by which hypoxia induces the development of the adhesion phenotype is believed to involve endogenous production of O2°-. Indeed, scavenging O2°-, by SOD, restores the adhesion phenotype markers, transforming growth factor-β1 and type I collagen, levels in adhesion fibroblasts to levels observed in normal peritoneal fibroblasts (12). Furthermore, scavenging O2. during hypoxia exposure protected against the development of the adhesion phenotype. Normal peritoneal fibroblasts treated with SOD combined with hypoxia prevented acquisition of the adhesion phenotype (12). Collectively, we have unequivocal evidence to support hypoxia as a result of tissue injury to be the cause of several profibrotic disorders, including postoperative adhesion.
- 11. Awonuga AO, Belotte J, Abuanzeh S, Fletcher NM, Diamond MP, Saed GM. Advances in the Pathogenesis of Adhesion Development: The Role of Oxidative Stress. Reprod Sci. 2014 Jul;21(7):823-836. Review. PMID: 24520085; PMCID: PMC4107571.

12. Fletcher NM, Jiang ZL, Diamond MP, Abu-Soud HM, Saed GM. Hypoxia-generated superoxide induces the development of the adhesion phenotype. Free Radic Biol Med. 2008;45:530-6. PMID: 18538674; PMCID: PMC2574925.

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- 4) Ex vivo postoperative adhesion model. Recently, we have established an ex vivo model that provides important information on the pathophysiology of adhesion development. First, it demonstrates that grossly visible adhesive bands can form within 24 hours, and that at this early time point it can already be dense and opaque (as opposed to filmy and transparent) (13). Secondly, consistent with clinical teaching, the placement of a blood clot between peritoneal surfaces incited greater incidence, tissue bloc surface area involvement, and tenacity of the adhesion band(s) that developed (13). Thus, these findings are consistent with the concept of attempting to achieve hemostasis to minimize adhesion development. Thirdly, the incidence, surface area of involvement, and tenacity of the adhesions that developed were greater following tissue traumatization (simulating tissue injury such as grasping with manipulation during the surgical procedure). Overall, this ex vivo peritoneal strip adhesion model provides a potentially valuable approach to examine efficacy of postoperative adhesion adjuvants as well as the study of the molecular biologic processes underlying development of adhesions (13).
- Saed GM, Fletcher NM, Diamond MP. The Creation of a Model for Ex Vivo Development of Postoperative Adhesions. Reproductive Science. Reprod Sci. 2016 May;23(5):610-2. PMID: 26408397.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1xiXzcfGjF7Q5/bibliography/46200971/public/?sort=date&direction=ascending

#### D. **Research Support**

Previous Research Support

**Prevent Cancer Foundation** 

Nicole M. King (PI)

01/16-01/18

Postdoctoral Fellowship Grant

### Novel biomarkers for early detection of ovarian cancer

The major goal of this project is to determine whether myeloperoxidase and free iron can be utilized as biomarkers for the early detection of ovarian cancer alone and in conjunction with currently available screening tools.

Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

#### **BIOGRAPHICAL SKETCH**

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Robert T. Morris, M.D.

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Professor, Program Director

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Saint John's University Callegaville Minnesste	Deshalanaf	1004	Ob a resistant
Saint John's University Collegeville, Minnesota	Bachelor of Science	1984	Chemistry
University of Minnesota, Minneapolis, Minnesota	Doctor of Medicine	1988	Doctor of Medicine
Linksonsky of Michigan Assa Askas Michigan	iviedicine		
University of Michigan, Ann Arbor, Michigan		1990-1991	Cellular and Molecular
			Biology
			Doctorate Program
Wayne State University, Detroit, Michigan		1991-1992	Cancer Biology
		.55. 1002	Doctorate Program
Wayne State University/Detroit Medical Center,	Docidonou	1004	
	Residency	1994	Obstetrics and
Detroit, Michigan			Gynecology
T 11 ' ' ' ' T 10 ' ' ' O	Fellowship	1996	Gynecologic Oncology
The University of Texas MD Anderson Cancer			
Center			

#### A. Personal Statement

I am a professor of gynecologic oncology, and my responsibilities include caring for patients with gynecologic malignancies, training residents and fellows, and advancing the knowledge in my field through research. I am well suited for participation and leadership in the research program because of my training, my research experience and production, as well as my contribution to education. In addition to two years of basic science post-graduate education at the University of Michigan and Wayne State University, I graduated from a very rigorous fellowship at the MD Anderson Cancer Center. I have been an institutional PI for over 30 studies at Wayne State University, and have been the PI on two investigator initiated multi-institutional clinical trials (1,2) As the director of Gynecologic Oncology, I am committed to overseeing the scientific efforts, the educational mission, and the medical management of patients with gynecologic malignancies. My research efforts include mentoring and directly participating with fellows in bench research as well as direct development, supervision, and participation in clinical research. In recognition of these qualifications and activities I have been a member of the Ovary Committee of the Gynecologic Oncology Group as well as contributed invited editorials from impactful journals (3).

- 1. Morris RT, Alvarez RD, Andrews S, Malone J, Bryant CS, Heilbrun LK, Schimp V, Munkarah AR. Topotecan weekly bolus chemotherapy for relapsed platinum-sensitive ovarian and peritoneal cancers. Gynecol Oncol. 2008. 109. 346-352.
- 2. Morris RT, Joyrich RN, Naumann RW, Shah NP, Maurer AH, Strauss HW, Uszler JM, Symanowski JT, Ellis PR, Harb WA. Phase II study of treatment of advanced ovarian cancer with folate-receptor-targeted

therapeutic (vintafolide) and companion SPECT-based imaging agent (99m Tc-etarforatide). Ann Oncol. 2014 Apr;25(4):852-8.

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3. Morris RT, Monk BJ. Ovarian cancer: relevant therapy, not timing, is paramount. Lancet. 2010 Oct 2;376(9747):1120-2.

#### **B.** Positions and Honors

Positions and E	mployment .
2005 - 2008	Wayne State University School of Medicine, Department of Obstetrics and Gynecology,
	Division of Gynecologic Oncology, Interim Director, Detroit, Michigan
2005 - Present	Wayne State University School of Medicine, Department of Obstetrics and Gynecology,
	Division of Gynecologic Oncology, Director, Fellowship Program, Detroit, Michigan
2006 – 2009	Wayne State University School of Medicine, Department of Obstetrics and Gynecology,
	Division of Gynecologic Oncology, Director, Detroit, Michigan
2009 - Present	Wayne State University School of Medicine, Department of Obstetrics and Gynecology,
	Division of Gynecologic Oncology, Interim Director, Detroit, Michigan
2009 - Present	Karmanos Cancer Institute, Wayne State University School of Medicine, Gynecologic
	Oncology Multidisciplinary Team Leader, Detroit, Michigan
2016 - Present	Wayne State University School of Medicine, Department of Obstetrics and Gynecology,
	Division of Gynecologic Oncology, Maria E. Brasza Endowed Chair in Gynecologic
	Oncology

<u>Honors</u>	
1988	Minnesota Medical Foundation Scholarship
1990 – 1991	National Institute of Health Human Genetic Training Grant
1992	Felix Rutledge Fellowship, MD Anderson Cancer Center, Houston, Texas
1992 – 1994	Holden Research Fellowship Grant
1993 – 1994	Stevenson Award for Academic Excellence, Wayne State University, Detroit, Michigan
1994	Wayne State University Fellows and Residents Outstanding Research Award, Detroit,
	Michigan
2000	Wayne State University School of Medicine Excellence in Teaching Award,
Detroit, Michigan	
2001, 2002, 2016	American College of Obstetricians and Gynecologists Council on Resident Education in
	Obstetrics and Gynecology National Faculty Award
2004, 2016	St. John Hospital and Medical Center Instructor of the Year
2006 - 2016	Detroit's Top Docs: Hour Detroit Magazine
2014	St. John's University Alumni Achievement Award

#### C. Contribution to Science

1) Folate targeted imaging in ovarian cancer: Ovarian cancer is a highly lethal malignancy. Although it initially responds to cytotoxic chemotherapy, it ultimately recurs and becomes chemoresistant. Predicting response to subsequent therapy has been challenging. Folate receptors are over- expressed on a large proportion of ovarian cancer. In a large multi-institutional phase 2 study, I investigated vintafolide folate targeted imaging prior to treatment with a folate targeting agent (EC45). This study demonstrated that those patients with folate positive imaging responded better to the therapy. This study has been part of the basis for our institutions commitment to folate targeted therapy and imaging. Morris RT, Joyrich RN, Naumann RW, Shah NP, Maurer AH, Strauss HW, Uszler JM, Symanowski JT, Ellis PR, Harb WA. Phase II study of treatment of advanced ovarian cancer with folate-receptor-targeted therapeutic (vintafolide) and companion SPECT-based imaging agent (99m Tc-etarforatide). Ann Oncol. 2014 Apr;25(4):852-8.

- 2) Chemotherapy administration dosing and scheduling: Although drug dosage is rigorously established through preclinical and phase 1 studies, the optimal schedule is often determined on a more theoretic basis. Topotecan is a topoisomerase inhibitor indicated in the treatment of ovarian cancer. It was developed as a 5 day infusion. Due to to toxicity and early clinical observations that weekly administration of other cell cycle specific agents may maintain or improve activity and diminish toxicity I developed a multi-institutional trial evaluating another dosing schedule of topotecan. This study demonstrated lower than expected toxicity while still demonstrating anticancer activity. These data contributed to the basis for 2 subsequent phase 3 cooperative group trials. Morris RT, Alvarez RD, Andrews S, Malone J, Bryant CS, Heilbrun LK, Schimp V, Munkarah AR. Topotecan weekly bolus chemotherapy for relapsed platinum-sensitive ovarian and peritoneal cancers. Gynecol Oncol. 2008. 109. 346-352.
- 3) Surgery: Ovarian cancer is the third leading cause of cancer deaths among women in the united staes. Due to its late presentation and aggressive clinical behavior, aggressive surgery and chemotherapy are used in nearly all patients. I have participated clinical trials defining the role of aggressive surgery in the management of ovarian cancer and assisted in manuscript preparation of the study. Overall the study found that volume of residual disease following surgery for recurrent disease was a predictive factor for disease free survival. Currently the goal of secondary surgery is to attain the level of no residual disease. Disease extent at secondary cytoreductive surgery is predictive of progression-free and overall survival in advanced stage ovarian cancer: An NRG Oncology/Gynecologic Oncology Group study. Rose PG, Java JJ, Morgan MA, Alvarez-Secord A, Kesterson JP, Stehman FB, Warshal DP, Creasman WT, Hanjani P, Morris RT, Copeland LJ. Gynecol Oncol. 2016 Dec;143(3):511-515. doi: 10.1016/j.ygyno.2016.09.005.
- 4) Mentoring: Chemotherapy cures some patients with ovarian cancer. Although some develop life- long toxicities such as neuropathy or cognitive impairment, some will ultimately develop secondary malignancies such as leukemia or myelodysplastic syndrome. One of my fellows questioned me as to the risk of such secondary cancers, and because the existing data was irrelevant a largescale epidemiologic review of the SEER database was performed. The development, supervision, and authorship of this study was by my fellow Dr A Vay under my mentorship. I include this as my contribution as an example of sheparding trainees through the process of inquiry, investigation, and reporting. Vay A, Kumar S, Seward S, Semaan A, Schiffer CA, Munkarah AR, Morris RT. Therapyrelated myeloid leukemia after treatment for epithelial ovarian carcinoma: An epidemiological analysis. Gynecol Oncol. 2011 Aug 18

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Case 3:16-md-02738-MAS-RLS	Document 33111-33 PageID: 230798	Filed 08/22/24	Page 162 of 285  Human primary normal ovarien epith  FIEM [Cell Biologics] CATAL  Media: Epithelial Cell Medium/WKid
			Cell dose Curve
			1.Cell-dose curve  Prepare cell-dose curve and time zero samples. Measure absorbance
			Cell-dose curve:  1). On day 0, prepare a cell-dose curve by using the stock (1 cells/ml in 1 X DMEM/10% FBS medium).  2). Prepare Blank (1X DMEM/10% FBS, no cells) and seven s
			dilutions of cells (2-fold) in separate 1.5 mL centrifuge tubes  1X DMEM/10% FBS as diluent.  Perform Serial Dilution by  a. Using an 8 channel multi-pipette, add 150 µl of m
			<ul> <li>each well of a 96 well microplate.</li> <li>b. Add 150 μl of 5.34x10<sup>s</sup> cells /ml (80x10<sup>3</sup> cells) soluthe wells of first duplicate row.</li> <li>c. Take 150 μl from the first duplicate row, add it to well and mix. The process is repeated as blow picture.</li> </ul>
	(2)		<ul> <li>Reserve the final well for the negative control (Blank well should contain media only (no cells).</li> <li>Also make a Test Cells(30K cells/well)</li> <li>Total volume will be 150 µL of each mixture into a selection.</li> </ul>
			well of a 96-well clear plate.  3). Add 35 µL of 1X DMEM/10% FBS and 15 µL of WST Work Solution into each well (Blank, Cell Standard Curve and Test 4) incubate at 37°C in an incubator for 4 hours.
			5). Measure absorbance using a microtiter plate reader at 4
2			

Case 3:16-n#LATEZEMPLATEAS-RLS DO

1 2 3 4 5 6 7 8 9 10 11 12

A 40k

B 20k 34k

C 10k

D 5k

E 25k

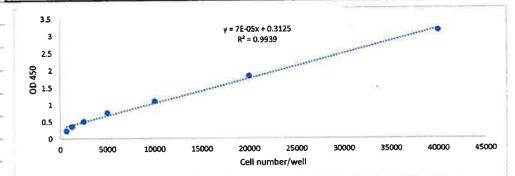
F 1.5k

G 7.45k

H 21.4k

# Results:

Standard with	PBS as Diluent						
	Cell Number	OD1	OD2	Average	Corrected 1	Corrected 2	Average
A	40000	3.9603	4.1406		3.05695	3.23725	3.1471
В	20000	2.7654	2.6965		1.86205	1.79315	1.8276
C	10000	2.0421	1.966		1.13875	1.06265	1.1007
D	5000	1.6562	1.6711		0.75285	0.76775	0.7603
E	2500	1.3806	1.4131		0.47725	0.50975	70.50975
F	1250	1.2585	1.2606		0.35515	0.35725	0.35725
G	675	1.1542	1.1118		0.25085	0.20845	0.22965
Blank	0	0.9156	0.8911	0.90335	0.9156	0.8911	0.90335
Test sample	31904	3.3764	3.5218		2.47305	2.61845	2.54575

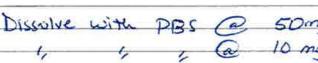


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PageID: 230799 Taleum pouder ( J&J. baby pour

# 30027477 , lot # 13





Social 3 times for Imin en Social Dismembrator (Fisher Se

For 100mm dishes will a 100 pl of 50mg/me to give 100 pl of 10 mg/me to give 100 pl of PBS as con

*

cells were seeded @ adensity of 30,000 as follows:

Cell Biologics

Blank

S Document 33111-33 Page 15:230800 Page)1@profe20@der

Filed 08/22/24

- To make a 1.2% solution of Agarose-powder: add 20 m
   Agarose into the Agarose Powder bottle
- Slightly open the bottle cap and place it on a heat bloc powder is entirely dissolved, gently shake the bottle to
- Move that bottle to a water bath of 37°C for 30 minut equilibrium.
- The 1.2% of agarose solution that isn't used can be sto conditions.
- 2) Preparation of DMEM solution (1 x DMEM plus 10 FB
- DMEM (10x)
- Water
- FBS (Hyclone Cat # SH30396.03, Lot# AD19958293)

Store at 4°C, but before using warm it up to 37°C in a wat

- 3) Preparation of WST reagent and electro coupling sol
- Add 1.8 mL of Electro Coupling Solution to the WST Re Working Solution.
- For short term storage place 4°C for 6 months, for lon and protect it from light.
- 4) Staining solution
  - Ready to use as supplied

### Assay Procedure

1) Preparation of Garded Agents as MAS-RLS Document 33111-33

-Prepare 75  $\mu L$  per well of base agarose mix as follows: PageID: 230801

1 Well:	
Component	Volume (µL)
1.2% Agarose solution	37.5
DMEM Solution (10X)	7.5
FBS	7.5
dH <sub>2</sub> O	22.5

12	We	lls	for	5	sam	ple
----	----	-----	-----	---	-----	-----

Component	Volume (µL)*
1.2% Agarose solution	450
DMEM Solution (10X)	90
FBS	90
dH <sub>2</sub> O	270

<sup>\*</sup>To account for error/extras we multiplied by 1.17

- Add 75 µL of the base agarose mix into required wells in the 96 well clear bottom tissue
- Keep the plate at 4°C for 15 minutes to solidify the agarose.

#### 2) Preparation of top Agarose layer with cells

- Use 30000 cells per well ( 20 μL of 1.5 x 10<sup>6</sup> cells/mL)
- Preparation of 75 μL/well mix of top Agarose as follows:

Component	1 well (µL)	12 wells ((µL) *
1.2% Agarose solution	25.0	300
DMEM Solution (10X)	5.5	66
FBS	5.5	66
Cells in 1XDMEM/10% FBS	20	240
dH <sub>2</sub> O	19	228

<sup>\*</sup>To account for error all of the 12 wells will be multiplied by 1.12

- Mix it in by using a pipette
- Add 75 µL of agarose-cell mix to wells to the base Agarose 96 well plate (step 1).
- Place the plate at 4°C for 10 minutes to solidify the layer.
- Then bring the plate back down to room temperature by placing it in the tissue culture hood for 10 minutes.
- Add 100 μL of 1X DMEM/10% FBS medium with and without talcum powder and incubate at 37°C for 7 days.
- Remove the upper medium on the top agarose layer by pipetting.
- Add 35 µL of 1X DMEM/10% FBS and 15 µL of WST Working Solution into each well.
- Incubate for 4 hours at 37°C and then using a microtiter plate reader measure the absorbance at 450 nm.

esults: Filed 08/22/24

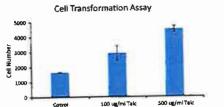
Page 165 of 285

8/5/2019 Sweded 30K/well Normal Ovarian Epithelial cells

6/12/2019 Temperature(\*C)

24.8 450 00

			1			cell	call .	
Treatment (ug/ml)	001	002	Average	Corr 001	Cert OD2	number 1	number 2	Average
Blank	0.98	0.94	0.96					-
Control	1.38	1.39	1.38	0.43	0.43	1615.71		-
100 ug/mi Tek	1.44	1.51	1	0.48	0.55	2441.43	3400.00	2920.7
500 ug/mi Telc	1.57	1.60		0.61	0.64	4314.29	4708.57	4511.4



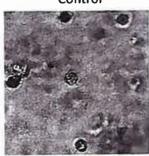
10.00% 8.00%

Cells were successfully transfor

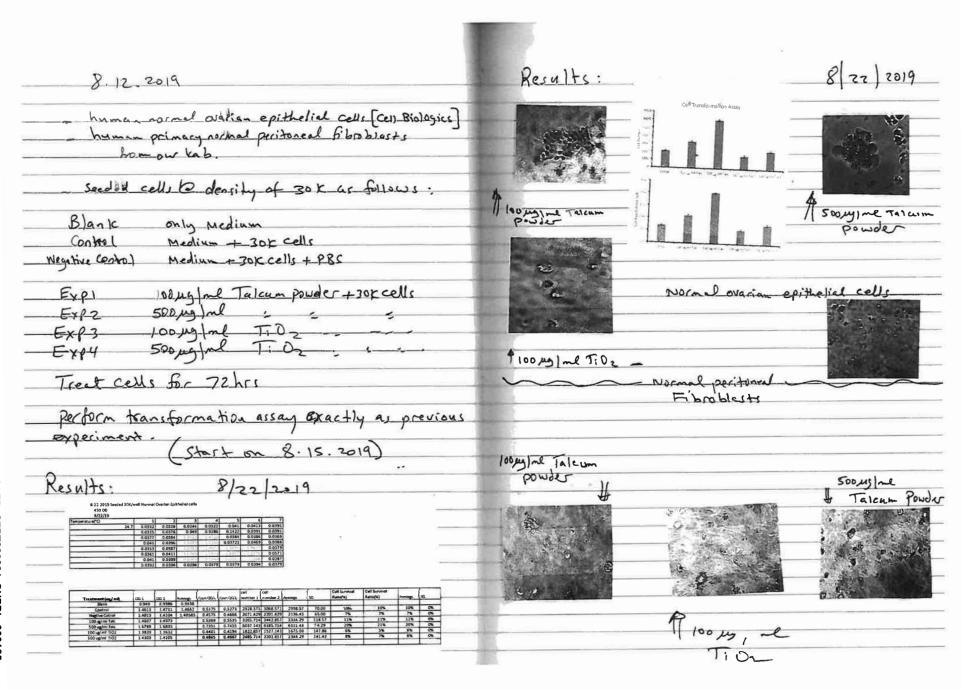
Normal Ovarian Cells

Control

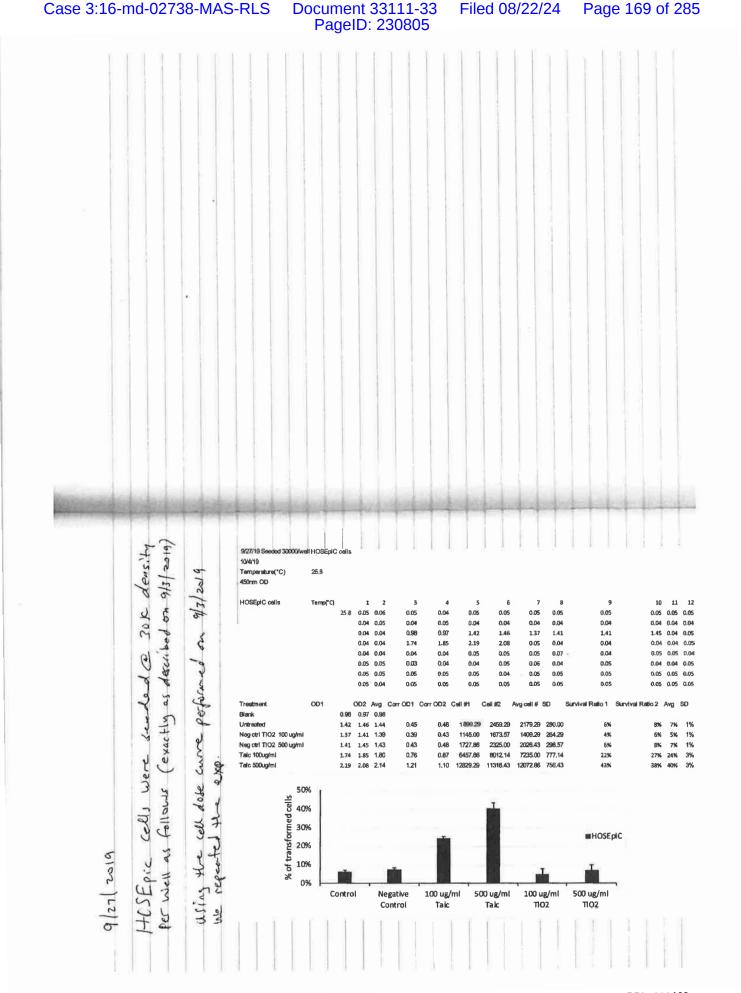
100 ug/ml Talc







	1/3/2019 465Epic		s Were	Cued	1 ed (a	2 Mens	its of 3	ak calle		27 / 7										
	viell as						17-7	or cety	Seeded 30000/well HO 9/27/19 Femperature(*C)	SEpiC cells 25.8										-
Uni	an 15 treated			ex d	actly	es des	cribed in	exp.	450nm OD HOSEpiC	Temp(°C) 25.8	1 0 0	2 0 0 0 0,887	3 4 1 0.7866	<b>5</b>	1.3544	1,2561	8.	1,3307	1.362	0
	02 100 m		2		Ιn	briplic	cate				0 0 0 0	0 1.81 0 0.794 0 1.977 0 0.834 0 2.009	0.8553 8 2.111 3 0.8122	2.3021 1.2784 2.4337 1.2591 2.3317	2.3772 1.2881 2.3888 1.2673 2.3877	1,3113	1.3411	1,2259 1,3112	1.273 1.32	0
Ta		1	ml_	T.	reat c	cells wi Zhrs.	th Talcum	powder	Traditment Blank Untreated Neg chi TiO2 100 ug/m Neg chi TiO2 500 ug/m Talc 100ug/mi Talc 500ug/mi	0.89 1.33 I 1.26	0.79 ( 1.35 1 1.28 1 1.36 1 1.79 1	Cont OD 84 34 0.5 27 0.4 35 0.4 80 0.9 34 1.4	2 0.44 9 0.53 7 0.96	1403.57 319.29 1385.00 8246.43	1723.57 635.00 1835.00 7979.29 16335.00		160.00 157.86 225.00 133.57	Survival Ratio 1 5% 1% 5% 27% 51%	69 29 69 279	Avg S 5% 1 5% 1 5% 1 27% 0
			4 S									Corr OD	a ann	0-11-#4					Survival Ratio 2	
Cel preu Ho	ll dose lionsly SEpic	deece cell	e Wa ined	is po	xform 81	ned e 11/201	q using		Trootment Blank Untreated Neg ctrl TiO2 100 ug/m Neg ctrl TiO2 500 ug/m Talc 100ug/ml Talc 500ug/ml	0.79 1.28 1.31	0.86 0 1.29 1 1.34 1 1.27 1 2.11 2	82 28 0.4 33 0.4 25 0.4	5 0.48 9 0.52 0 0.45 5 1.29	811.43 1281.43 61.43 10802.86	950.00 1707.14 738.57 12706.71	880.71 1494.29 400.00 11754.29 16995.00	69.29 212.86 338.57 951.43	Survival Ratio 1 3% 4% 0% 36% 58%	39 69 29 429	3% 5 5% 1% 39% 3
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12.8.2020
Two pormal ovarian epithelial cell lives
- Human primary normal ovarian epithelial cells (cell Biologicia) - Human ovarian sexisface epithelial cells HOSEPIC (sciental)
( science)
- Cells were grown in 100 mm plate and treated with
100 mg/ml talcum powder see methods described
0~ 812/2019
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- Cells were siren to WSO, Separtment of Pathology to perform Formunahistochemistry
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1. 2. 2002 6 3 1
NOEC DAPT CONTOL
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NO EC BS3 : .
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HOSEPIC Ki67 :
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HOSE PIC PS3 & = =
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Table 1	2	0 0	The same of the sa
Antibodies and staining conditions for immunohistochemistry	OE OE	0 00	e .
Antibody Clone Source Detection system Dilution P53 DO-7 Ventana Ventana ultraView DAB 1:500 Ki-67 Mib1 Ventana Ventana ultraView DAB 1:2000  Cytospin slides prepared from cell line cultures and stained (image1) using	NOEC-Control		
immunoperoxidase labeling performed with the automated XT iVIEW DAB V.1 procedure on the Ultra BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone DO-7 prediluted, Ventana). Antigen retrieval was carried out		Diff quick staln	Ki-67 (red): 5
with CC1, pH 8.0 (Ventana). Sections were incubated with primary antibodies for 36 min at 37°C. All slides were reviewed by two pathologists (RA and AA).  Cases with discordant Ki-67 estimated results underwent a consensus review at a double-headed microscope. Diffuse "in-block" nuclear staining or			
complete negative staining with p53 was considered a positive reaction indicating mutated p53 status. Focal nuclear staining is consistent with "wild type" p53 and considered negative. The Proliferation Index (PI) was assessed qualitatively using Ki-67 stained slides and classified as high PI (>50% positive cells) or low PI (<50% positive cell) [1].	NOEC-Taic		
Refrence	n		
[1] MAhAdevAPPA, AShA, Shruthi Mysore Krishna, and Manjunath Gubbanna Vimala.  "Diagnostic and prognostic significance of Ki-67 immunohistochemical expression in surface epithelial ovarian carcinoma." Journal of Clinical and Diagnostic Research:  JCDR 11.2 (2017): EC08.		Diff quick stain	Ki-67 (red): 70 (high prolifera
X			
	1		

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Document 33111-33 PageID: 230808

Case 3:16-md-02738-MAS-RLS

Filed 08/22/24

Page 172 of 285

# **Invoice**

**DS Biotech, LLC** 

1665 Dell Rose Bloomfield Hills, MI 48302 Ghassan Saed

Bill To: Beasley Allen **Beasley Allen** 218 Commerce St Montgomery, AL 36104 Date: 07/14/2020 Invoice No.: 10063 Due Date: 07/24/2020

Qty	Item	Description	Unit Price	Total
65	Consulting	Consulting	\$600.00	\$39,000.00

Document 33111-33 PageID: 230809

> Total \$39,000.00 Balance Due \$39,000.00

Please contact us for more information about payment options.

Thank you for your business.



## **DS Biotech, LLC**

1665 Dell Rose Bloomfield Hills, MI 48302 248-894-1474 gsaed@dsbiotech.net

INVOICE

INV0004

DATE Jan 5, 2021

DUE

On Receipt

**BALANCE DUE** 

USD \$27,600.00

**BILL TO** 

## **Beasley Allen**

218 Commerce st Montgomery, Al 36104 3345465435 leigh.odell@BeasleyAllen.com

DESCRIPTION	RATE	QTY	AMOUNT
Consultation	\$600.00	46	\$27,600.00
	SUBTOTAL		\$27,600.00
	TAX (0%)		\$0.00
	TOTAL		\$27,600.00
	BALANCE DUE	USD \$	27,600.00

From: McKay, Samantha (HC/SC) samantha.mckay@canada.ca

Subject: RE: A-2018-001795 Date: June 24, 2020 at 11:00 AM

To: Ghassan Saed gsaed@med.wayne.edu



## Hello.

The request is for records in relation to the Draft Screening Assessment – Talc and Health Canada's communication with you regarding it. The records pertaining to yourself specifically is a few emails and a published document (31 pages in total). I would need you to review the records and identify any information you feel should not be released to the public.

Document 33111-33

PageID: 230811

## Regards,

Samantha McKay Consultant Access to Information & Privacy Health Canada and Public Health Agency of Canada / Government of Canada samantha.mckay@canada.ca

## Samantha McKay Consultante

Accès à l'information et de la protection des renseignements personnels Santé Canada et Agence de la santé publique du Canada / Gouvernement du Canada samantha.mckay@canada.ca

From: Ghassan Saed <gsaed@med.wayne.edu>

Sent: 2020-06-24 10:54 AM

To: McKay, Samantha (HC/SC) <samantha.mckay@canada.ca>

Subject: Re: A-2018-001795

What kind of information they are requesting?

Best Regards Dr. Ghassan Saed, Associate Professor Department of OB/GYN Wayne State University Medical School Detroit, MI

> On Jun 24, 2020, at 2:34 AM, McKay, Samantha (HC/SC) <samantha.mckay@canada.ca> wrote:

TT.11.

пено,

I hope this email finds you well. I am processing the above noted ATIP request and have identified some records that pertain to you, I will need to consult with you prior to releasing them to the requester.

As we are currently unable to utilise mail/courier services, I will need to send them via email. If you could confirm receipt of this email in a timely manner it would be greatly appreciated.

Thank you in advance for your assistance,

Samantha McKay Consultant Access to Information & Privacy Health Canada and Public Health Agency of Canada / Government of Canada samantha.mckay@canada.ca

Samantha McKay Consultante Accès à l'information et de la protection des renseignements personnels Santé Canada et Agence de la santé publique du Canada / Gouvernement du Canada samantha.mckay@canada.ca

From: Praine, Lisa (HC/SC) lisa.praine@canada.ca

Subject: RE: Access to Information Consultation from Health Canada - A-2018-001795

Date: December 8, 2020 at 8:48 AM

To: Ghassan Saed gsaed@med.wayne.edu

#### Good morning,

I just wanted to follow up on the consultation that we sent to you. Please let me know when we can expect your response (by email is fine).

Document 33111-33

PageID: 230813

Feel free to call or email me if you want to discuss.

Thank you,

#### Lisa Praine

Manager, Access to Information and Privacy Health Canada and Public Health Agency of Canada / Government of Canada lisa.praine@canada.ca / Tel: 343-542-5320 / Fax: 613-941-4541

Gestionnaire, Accès à l'information et protection des renseignements personnels Santé Canada et Agence de la santé publique du Canada / Gouvernement du Canada lisa.praine@canada.ca / Tél: 343-542-5320 / Télécopieur 613-941-4541

From: Ghassan Saed <gsaed@med.wayne.edu>

Sent: 2020-10-22 1:46 PM

To: Praine, Lisa (HC/SC) < lisa.praine@canada.ca>

Subject: Re: Access to Information Consultation from Health Canada - A-2018-

001795

Hi Lisa.

I have created an account for Epost that is linked to email gsaed87@yahoo.com. Best regards Ghassan Saed

On Oct 13, 2020, at 1:39 PM, Praine, Lisa (HC/SC) lisa.praine@canada.ca > wrote:

#### [EXTERNAL]

Good afternoon.

I work in the Access to Information and Privacy Division of Health Canada. I am currently working on an Access to Information request and I have come across some documents that originated with your organization. I am preparing a consultation to send to your for your recommendations as to their release to our applicant.

Given our current work conditions (working from home), I am wondering if you would be willing to sign up for EPOST, at no cost to you, so that I could send the records to you securely. Sending information via EPOST is

much more secure than sending it by regular email. Please let me know if/when you are willing to set up and EPOST account and to which email address I can send the records in EPOST. Please find attached a document with more information about EPOST.

If you would prefer to receive the documents by courier, please provide me with your complete mailing address.

I await your response.

Please feel free to call or email me if you have any questions.

Thank you,

#### Lisa Praine

Manager, Access to Information and Privacy Health Canada and Public Health Agency of Canada / Government of Canada lisa.praine@canada.ca / Tel: 343-542-5320 / Fax: 613-941-4541

Gestionnaire, Accès à l'information et protection des renseignements personnels Santé Canada et Agence de la santé publique du Canada /

Gouvernement du Canada

lisa.praine@canada.ca / Tél : 343-542-5320 / Télécopieur 613-941-4541

<epost poster HC-SC.docx>

Dr. Ghassan M. Saed

Associate Professor of Gynecologic Oncology

Director of Ovarian Cancer Biology Research

Departments of Obstetrics and Gynecology and Oncology

Member of Tumor Biology and Microenvironment Program

Karmanos Cancer Institute

Wayne State University School of Medicine

Detroit, MI 48202

(313) 577-5433 Office

(313) 577-1302 Lab

(313) 577-4633 Fax

Date of Preparation: September 16, 2021

Signature

# GHASSAN M. SAED, Ph.D. **Associate Professor with Tenure (Research)**

**OFFICE ADDRESS:** The C.S. Mott Center for Human Growth and Development

Department of Obstetrics and Gynecology

275 East Hancock Avenue

Detroit, MI 48201

**OFFICE TELEPHONE NUMBER:** (313) 577-5433

**OFFICE FAX NUMBER:** (313) 577-8554

**EMAIL ADDRESS:** g.saed@wayne.edu

### **EDUCATION:**

Ph.D. in Molecular Biology University of Essex, Colchester, England, United Kingdom	1983–1986
B.S. in Biochemistry King Saud University, Riyadh, Saudi Arabia	1979–1982
POSTGRADUATE TRAINING:	
Fellowship in Immunopathology, University of Michigan, Ann Arbor, MI Fellowship in Molecular Biology, Henry Ford Hospital, Detroit, MI	1992–1993 1988–1990
FACULTY APPOINTMENTS:	
Adjunct Associate Professor, Department of Oncology, Karmanos Cancer Institute, Detroit Medical Center/Wayne State University School of Medicine, Detroit, MI	2017-Present
Director, Ovarian Cancer Biology Research, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI	2009-Present
Scientific Member, Karmanos Cancer Institute, Molecular Biology and Genetics Program, Wayne State University School of Medicine, Detroit, MI	2008-Present
Member of Tumor Biology and Microenvironment Program, Karmanos Cancer Institute, Detroit, MI	2008-Present
Associate Professor (secondary), Department of Physiology, Wayne State University School of Medicine, Detroit, MI	2008-Present

Associate Professor (primary), Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Tenure, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Associate Status, Department of Anatomy/Cell Biology, Wayne State University School of Medicine, Detroit, MI Tenure Track, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Tenure Track, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Assistant Professor, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI HOSPITAL OR OTHER PROFESSIONAL:  Senior Investigator, Center for Biomedical Research, Oakland University, Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Associate Staff Investigator, Dermatology Department, Henry Ford Hospital, Detroit, MI Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI Assistant Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology Member, American Federation of Clinical Research Member, American Society for Reproductive Medicine Member, American Society for Reproductive Investigation Member, American Society for Reproductive Investigation Member, American Council of the United Kingdom Member, American Society for Reproductive Investigation Member, American Council of the United Kingdom Member, American Society for Reproductive Medicine Michigan State University in the laboratory		
Tenure, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Associate Status, Department of Anatomy/Cell Biology, Wayne State University School of Medicine, Detroit, MI Tenure Track, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Assistant Professor, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Assistant Professor, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI  HOSPITAL OR OTHER PROFESSIONAL:  Senior Investigator, Center for Biomedical Research, Oakland University, Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Associate Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology Member, American Association of Cancer Research Member, American Society for Reproductive Medicine Member, American Society for Reproductive Medicine Member, American Chemical Society Mem		2007-Present
Associate Status, Department of Anatomy/Cell Biology, Wayne State University School of Medicine, Detroit, MI Tenure Track, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Assistant Professor, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Assistant Professor, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI HOSPITAL OR OTHER PROFESSIONAL:  Senior Investigator, Center for Biomedical Research, Oakland University, Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Bioscientific Staff Investigator, Dermatology Department, Henry Ford Hospital, Detroit, MI Associate Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology Member, American Association of Cancer Research Member, American Society for Reproductive Medicine Member, American Society for Reproductive Medicine Member, American Chemical Society Member, Society for Reproductive Medicine (ASRM) Scientific Member, Soc	Tenure, Department of Obstetrics and Gynecology, Wayne State University	2007-Present
Tenure Track, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI Assistant Professor, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI  HOSPITAL OR OTHER PROFESSIONAL:  Senior Investigator, Center for Biomedical Research, Oakland University, Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Bioscientific Staff Investigator, Dermatology Department, Henry Ford Hospital, Detroit, MI Bioscientific Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, American Association of Cancer Research Member, American Association of Cancer Research Member, American Society for Reproductive Medicine Member, American Association of University Professors Member, American Association of University Professors Member, American Chemical Society Member, American Chemica	Associate Status, Department of Anatomy/Cell Biology, Wayne State	2003-Present
Assistant Professor, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI  HOSPITAL OR OTHER PROFESSIONAL:  Senior Investigator, Center for Biomedical Research, Oakland University, Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Bioscientific Staff Investigator, Dermatology Department, Henry Ford Hospital, Detroit, MI Associate Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology Member, American Association of Cancer Research Member, American Federation of Clinical Research Member, American Federation of Clinical Research Member, American Association of University Professors Member, American Chemical Society National Research Council of the United Kingdom Medical Research Council of the United Kingdom Medical Research Council of the United Kingdom HONORS/AWARDS: Oncology Research fellowship Em Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.  Star Award 73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo This award recognizes members who have presented during at least nine	Tenure Track, Department of Obstetrics and Gynecology, Wayne State	2001–2007
Senior Investigator, Center for Biomedical Research, Oakland University, Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Bioscientific Staff Investigator, Dermatology Department, Henry Ford Hospital, Detroit, MI Associate Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology Member, American Association of Cancer Research Member, American Society for Reproductive Medicine Member, American Society for Reproductive Medicine Member, American Chemical Society Member, American Society for Reproductive Medicine Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Council of the United Kingdom  Medical Research Coun	Assistant Professor, Department of Obstetrics and Gynecology, Wayne	1998–2007
Rochester, MI Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI Bioscientific Staff Investigator, Dermatology Department, Henry Ford Hospital, Detroit, MI Associate Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology 2017—Present 2008—Present Member, American Association of Cancer Research 2008—Present Member, American Federation of Clinical Research 2009—Present Member, American Society for Reproductive Medicine 1998—Present Member, American Association of University Professors 1996—Present Member, American Chemical Society 1998—Present 1998—Present 1991—2014 National Research Council of the United Kingdom 1985—1986 Medical Research Council of the United Kingdom 1984—1998  HONORS/AWARDS: Oncology Research fellowship 2017  Emi Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.  Star Award 73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo This award recognizes members who have presented during at least nine	HOSPITAL OR OTHER PROFESSIONAL:	
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Associate Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI  Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI  Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology Member, American Association of Cancer Research Member, American Federation of Clinical Research Member, American Society for Reproductive Medicine Member, Society for Reproductive Investigation Member, American Association of University Professors Member, American Chemical Society Medical Research Council of the United Kingdom Medical Research Council of the United Kingdom Medical Research Council of the United Kingdom  HONORS/AWARDS: Oncology Research fellowship Emi Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.  Star Award 73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo This award recognizes members who have presented during at least nine	Bioscientific Staff Investigator, Dermatology Department, Henry Ford	1995–1998
Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI  Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology Member, American Association of Cancer Research Member, American Federation of Clinical Research Member, American Society for Reproductive Medicine Member, Society for Reproductive Investigation Member, Society for Reproductive Investigation Member, American Association of University Professors Member, American Chemical Society Member, American Chemical Society National Research Council of the United Kingdom Medical Research Council of the United Kingdom Medical Research Council of the United Kingdom  HONORS/AWARDS: Oncology Research fellowship Emi Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.  Star Award  2017  Star Award 73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo This award recognizes members who have presented during at least nine	Associate Staff Investigator, Department of Dermatology, Henry Ford	1993–1994
Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI  MAJOR PROFESSIONAL SOCIETIES  Member, Society for Gynecologic Oncology Member, American Association of Cancer Research Member, American Federation of Clinical Research Member, American Society for Reproductive Medicine Member, American Society for Reproductive Medicine Member, American Association of University Professors Member, American Association of University Professors Member, American Chemical Society Member, American Chemical Society National Research Council of the United Kingdom Medical Research Council of the United Kingdom HONORS/AWARDS: Oncology Research fellowship Emi Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.  Star Award 73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo This award recognizes members who have presented during at least nine	Special Lecturer, Department of Chemistry, Oakland University, Rochester,	1991–1996
Member, Society for Gynecologic Oncology Member, American Association of Cancer Research Member, American Federation of Clinical Research Member, American Federation of Clinical Research Member, American Society for Reproductive Medicine Member, Society for Reproductive Investigation Member, American Association of University Professors Member, American Chemical Society Member, American Chemical Society Member, American Chemical Society Metional Research Council of the United Kingdom Medical Research Council of the United Kingdom Medical Research Council of the United Kingdom  HONORS/AWARDS: Oncology Research fellowship Emi Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.  Star Award 73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo This award recognizes members who have presented during at least nine	Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital,	1990–1992
Member, American Association of Cancer Research Member, American Federation of Clinical Research Member, American Society for Reproductive Medicine Member, Society for Reproductive Investigation Member, American Association of University Professors Member, American Association of University Professors Member, American Chemical Society National Research Council of the United Kingdom Medical Research Council of the United Kingdom Medical Research Council of the United Kingdom  HONORS/AWARDS: Oncology Research fellowship Emi Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.  Star Award 73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo This award recognizes members who have presented during at least nine	MAJOR PROFESSIONAL SOCIETIES	
Oncology Research fellowship  Emi Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.  Star Award 73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo This award recognizes members who have presented during at least nine	Member, American Association of Cancer Research Member, American Federation of Clinical Research Member, American Society for Reproductive Medicine Member, Society for Reproductive Investigation Member, American Association of University Professors Member, American Chemical Society National Research Council of the United Kingdom	2008-Present 2009-Present 1998-Present 1998-Present 1996-Present 1991-2014 1985-1986
73 <sup>rd</sup> American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo  This award recognizes members who have presented during at least nine	Oncology Research fellowship <u>Emi Bulica</u> , awarded a year-off oncology research scholarship from  Michigan State University in the laboratory of Dr. Saed of the  Department of Obstetrics & Gynecology at Wayne State University.  This award is for 12 months of full-time research in Dr. Saed's laboratory	2017
of the ACRM Annual Maetings from the years 2007, 2016. Procentations	73 <sup>rd</sup> American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo  This award recognizes members who have presented during at least nine	2017

of the ASRM Annual Meetings from the years 2007-2016. Presentations

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2017

2015

may include Congress courses and/or seminars, Scientific Program symposia, posters, and/or oral presentation of abstracts. 234 awardees

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# SRS In-Training Award for Research Awarded to Nicole King, PhD, Postdoctoral Fellow in the laboratory of Dr. Ghassan Saed

73rd American Society for Reproductive Medicine (ASRM Scientific Congress & Expo

The purpose of this award is to recognize outstanding research conducted by individuals in training under the "Reproductive Surgery" category. He/she is a presenting first author, and a medical student, resident, fellow, or undergraduate, graduate, or postdoctoral student. Three awardees

Star Award 2016

72<sup>nd</sup> American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo

This award recognizes members who have presented during at least nine of the ASRM Annual Meetings from the years 2007-2015. Presentations may include Congress courses and or seminars, Scientific Program symposia, posters, and/or oral presentation of abstracts. ~235 awardees

## **Excellence in Biomedical Research**

Global Medical Discovery Series

Key Scientific Article for peer-reviewed publication entitled: "Sox2 Gene Amplification Significantly Impacts Overall Survival in Serous Epithelial Ovarian Cancer." Reproductive Sciences 22(1):38-46, 2015. Epub July 18, 2014. One awardee

2013 Star Award

69<sup>th</sup> American Society for Reproductive Medicine Annual Meeting (ASRM) This award recognizes members who have presented during at least nine of the ASRM Annual Meetings from the years 2007-2012. Presentations may include Congress courses and/or seminars, Scientific Program symposia, posters, and/or oral presentation of abstracts. ~235 awardees

**Star Award** 2011

67<sup>th</sup> American Society for Reproductive Medicine (ASRM) Annual Meeting This award recognizes members who have presented during at least nine Of the ASRM Annual Meetings from the years 2007-2010. Presentations may include Congress courses and/or seminars, Scientific Program symposia, posters, and/or oral presentation of abstracts. ~235 awardees

Award for Graduate Students Who Obtained External Support 2010

Jennell White, Obstetrics/Gynecology

Advisor: Ghassan Saed, Obstetrics/Gynecology

Agency: National Institutes of Health

#### President's Award for Excellence in Teaching 2009

Wayne State University School of Medicine

This award is in recognition for outstanding faculty who have made contributions to teaching at WSU to an exceptionally high degree, demonstrate comprehensive knowledge of their subject, superior classroom performance, and high educational standards; communicate their subject matter accurately, clearly, and effectively; generate enthusiasm and respect for learning; motivate their students to excel; and are accessible to students; innovative instructional practices, impact on teaching at WSU, and contributions to advancing teaching in their field.

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<b>Finalist Paper</b> 62 <sup>nd</sup> American Society for Reproductive Medicine (ASRM) Annual Meeting One awardee	2006
Travel Award winner 1st International Conference on Ovarian Cancer: State of the Art and Future Directions, Aegean conferences, Greece	2006
Prize Paper Candidate Conjoint 61st American Society for Reproductive Medicine (ASRM) Annual Meeting and 51st Canadian Fertility and Andrology Society Annual Meeting One awardee	2005
<b>Finalist Paper</b> 61st American Society for Reproductive Medicine (ASRM) Annual Meeting <i>One awardee</i>	2005
Finalist Paper Society of Reproductive Endocrinology and Infertility (SREI) Annual Meeting One awardee	2003
Finalist Paper, Basic Science 19 <sup>th</sup> European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting One awardee	2003
<b>Award Paper</b> 58 <sup>th</sup> Society of Reproductive Surgeons (SRS) Scientific Program	2000
Finalist Paper Society of Reproductive Endocrinology and Infertility (SREI) Annual Meeting One awardee	2000
<b>Award Paper</b> 52 <sup>nd</sup> Society of Reproductive Surgeons (SRS) Scientific Program	1998
Outstanding Professor of the Year Award Golden Key National Society, Oakland University Chapter, Rochester, MI One awardee	1996–1997

# **SERVICE:**

# **Wayne State University**

# **Departmental/Divisional**

Chairperson, Organizing Committee, 2017 Joint Annual Reproductive Sciences Retreat, Departments of Obstetrics and Gynecology, Wayne State University School of Medicine and University of Toronto; and The Michigan Alliance for Reproductive Technologies and Sciences (MARTS) Annual Meeting at Wayne State University	2017
Faculty Mentor, NIH/NICHD Women's Reproductive Health Research (WRHR) Scholar Program, Department of Obstetrics and Gynecology Faculty Associate, Fulbright Visiting Senior Scholar Award recipient Dr. Iyad Ali, Department of Obstetrics and Gynecology	2012–2016 2012–2014
Member, Selective Salary Committee, Department of Obstetrics and Gynecology	2012-Present
Member, Promotion and Tenure Committee, Department of Obstetrics and Gynecology	2012-Present
Chairperson, C.S. Mott Center Seminar Series Committee, Department of Obstetrics and Gynecology	1998-Present
Chairperson, Basic Research Endocrine Fellows Training Committee, Department of Obstetrics and Gynecology	1998–2014
Member, Reproductive Endocrinology and Infertility Fellowship Selection Committee, Department of Obstetrics and Gynecology	1998-Present
School of Medicine	
<u>Chair, Master committee for Osama Nusrat, MD, MS candidate</u> , Department of Physiology in the Reproductive Sciences Wayne State University School of Medicine.	2015-2017
Member, Strategic Research Initiative Grant Review (SRIG) Committee, Karmanos Cancer Institute	2013–2014
Member, PhD Committee for Batoul Abdullah, PhD Candidate, Center for Molecular Medicine and Genetics, Wayne State University	2012–2016
Chair, PhD Committee for Jimmy Belotte, MD, PhD Candidate, Department of Physiology and Reproductive Sciences, Wayne State University School of Medicine.	2012–2016
Faculty, Reproductive Sciences Graduate Program, Department of Physiology, Wayne State University	2012–2016

Chair, PhD committee for Nicole M. Fletcher, PhD candidate Department of Physiology and Reproductive Sciences, Wayne State University School of Medicine.	2008-2013
Chair, PhD committee for Jennell White Jackson, PhD candidate, Department of Physiology in the Reproductive Sciences, Wayne State University School of Medicine.	2000-2011
Member, Search Committee for a candidate selection for a joint appointment in the Departments of Psychology and Obstetrics & Gynecology in the field of Psychopharmacology	2003–2005

# **Affiliate Medical Organizations**

National Arab American Medical Association: Serve as Chief Financial Officer, 2009–Present Board Member, and two times President of Michigan Chapter.

#### **Professional**

Member for publication committee at the Society of Reproductive Investigation	2019-Present
Society for Gynecologic Oncology: Serve as judge for oral and poster presentations and abstract selection committee.	2017-Present
Karmanos Cancer Institute: Design research projects and give lectures in the Molecular Biology and Genetics Program.	2008-Present
Karmanos Cancer Institute: Design, discuss and present research projects for the Tumor Biology and Microenvironment Program.	2007-Present
Society for Reproductive Investigation: Serve as judge for oral and poster presentations and abstract review committee.	2005-Present
American Society for Reproductive Medicine: Serve as judge for oral and poster presentations, and abstract review committee.	2006-Present
Yale University, New Haven, CT: Served as a member in Scientific Review Committee, Ethel F. Donoghue Women's Health Investigator Program,	2004-2004
Henry Ford Hospital, Detroit, MI: Served in Biohazard and Safety Committee, Radioisotope Safety Committee, Animal Care Committee, Residents/Fellows Basic research training and education committee	1993-1998
Consulting	
	0047.5

Consultant – Collaborator for the Department of Obstetrics and Gynecology, 2017–Present University of Illinois, Chicago, IL

Consultant and Witness Expert on behalf of plaintiffs in national litigation 2017–Present

against Johnson & Johnson Baby Powder and increased risk of ovarian cancer	
Consultant – Collaborator for the Department of Obstetrics and Gynecology, University of Tennessee Health Science Center, Memphis, TN	2012-Present
Consultant – Collaborator for the Department of Obstetrics and Gynecology, University of Augusta, GA	2013-Present
Consultant, Molecular Biologic Testing, DS Biotech, Detroit, MI	2013-Present
Consultant, Application of Cyclooxgenase-2 in the Treatment of Ovarian Cancer, Pfizer Pharmaceuticals, Rochester, MI	2002-2003
Consultant, Technical expertise in developing molecular probes and Markers, Oxford Biomedical Research, Oxford, MI	1991–1998
Scholarly Service	
Grant Review Committees	
Member, Scientific Review Committee, Ethel F. Donoghue Women's Health Investigator Program, Yale University, New Haven, CT	2004
Service for Peer-Reviewed Journals Editorship	
Editorial Board Membership:	
Editor-in-Chief, Gynecology and Obstetrics Research-Open Journal	2015-Present
Review of Manuscripts and Chapters:	
Journal of Cellular and Molecular Medicine Systems Biology in Reproductive Medicine Journal of Assisted Reproduction and Genetics Journal of Reproductive Science European Journal of Obstetrics & Gynecology and Reproductive Biology Gastroenterology Houghton Mifflin Company, College Division American Gynecological and Obstetrical Society Oncogenes Fertility and Sterility Wound Repair and Regeneration Journal of Cytokine Research	2015–Present 2013–Present 2013–Present 2012–Present 2009 2007 2003 2003 2003 2001–Present 2000–Present 1998–2000

# **TEACHING**

# **Teaching at Wayne State University**

# <u>Undergraduate Students</u>

Instructor. Department of Biological Sciences – BIO 3990: Undergraduate course primarily for biology majors who wish to continue in a field beyond that covered in regular courses under the direction of Biological Sciences faculty.

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Instructor and Advisor. Department of Physiology – PSL 5010: Undergraduate course involving student participation in laboratory research in the physiological sciences under the supervision of a departmental faculty advisor.

This course involves an introduction to experimental protocol and current related scientific literature.

Advisor. Department of Biological Sciences – BIO 6990: Undergraduate course for honors students involving student participation in laboratory research in the physiological sciences under the supervision of a departmental faculty advisor.

#### **Graduate Students**

Interdisciplinary Biomedical Sciences - IBS 7060: Biomedical Endocrine and Instructor. Reproductive Systems Development.

This course is for graduate students within the Ph.D. Program in Anatomy and Cell Biology of which has the aim of providing a broad-based knowledge of the important areas of biomedical research.

Instructor. Department of Physiology with Concentration in the Reproductive Sciences Program (PhD) – RPS 7350: Biomolecular Techniques: From Genes to Protein

Instructor. Department of Physiology with Concentration in the Reproductive Sciences Program (PhD), Principles of Reproductive Biology – PSL 7690: Cancers in Reproductive Organs/ Journal Club.

This lecture explains the impact of cancer in women; to discuss the epidemiology, risk factors, screening modalities and preventative strategies of gynecologic cancers and the role of stem cells.

Instructor. Current Research Topics in the Reproductive Sciences – PSL 7775: Molecular Mechanisms of Postoperative Adhesions.

This course is for graduate students within the Ph.D. Program in Physiology with Concentration in the Reproductive Sciences of which covers current research topics in reproductive sciences. The Program itself incorporates the teaching, research and physical resources of both the Physiology and the Obstetrics and Gynecology Departments, offering interdisciplinary doctoral training in a clinical environment in the reproductive sciences. The primary academic focus engages teaching and research training in reproduction and development, with an emphasis on the following: developmental biology, perinatal biology, reproductive endocrinology, reproductive genetics, toxicology/teratology and molecular biology including genomics, proteomics, and bioinformatics. Dissertation research is under the mentorship of Obstetrics and Gynecology basic science graduate faculty.

Advisor and Mentor. Current Research Topics in the Reproductive Sciences – PSL 7996: Arranged Research.

This course is for the graduate students within the "Ph.D. Program in Physiology with Concentration in the Reproductive Sciences" (as described in PSL 7775) which covers graduate level experiences in research techniques. It is required that special research topics, within specified areas, be agreed upon between individual faculty members and students.

Doctoral Candidate Status I-IV - PSL 9991, 9992, 9993, 9994: Advisor and Mentor. Thesis/Dissertation Research and Design.

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This course is for the graduate students within the "Ph.D. Program in Physiology with Concentration in the Reproductive Sciences" (as described in PSL 7775). Required in consecutive academic-year semesters following advancement to Ph.D. candidacy status I through IV.

Advisor and Mentor. Doctoral Candidate Dissertation Research and Direction - PSL 9995: Candidate Maintenance Status.

This course is for the graduate students within the "Ph.D. Program in Physiology with Concentration in the Reproductive Sciences" as described above in PSL 7775. Required after completion of 30 credits in PSL 9991-9994.

<u>Director</u>. Summer Reproductive Technology Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2014-2015.

The course design is to allow for Reproductive Endocrinology and Infertility/Medical Genetics fellows, as well as graduate students to become familiar with laboratory techniques in the reproductive sciences. The graduate students will acquire a thorough understanding of the theory and special methodology utilized to perform techniques indicative of reproductive endocrinology and infertility.

Lecturer. Laboratory Techniques. Summer Reproductive Technology Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2014.

This lecture explains the various laboratory techniques, and their limitations, as applied to the reproductive sciences.

Lecturer. Molecular Biological Procedures. Summer Reproductive Technology Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2015.

This lecture explains the various laboratory techniques, and their limitations, as applied to the reproductive sciences.

#### Residents and Fellows

Director. Summer Reproductive Technology Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2014-2015.

The course design is to allow for Reproductive Endocrinology and Infertility/Medical Genetics fellows, as well as graduate students to become familiar with laboratory techniques in the reproductive sciences. The fellows will acquire a thorough understanding of the theory and special methodology utilized to perform techniques indicative of reproductive endocrinology and infertility.

Instructor. PCR Technique: Concept and Clinical Application Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2012-Present.

This course is designed to allow departmental residents, fellows, Reproductive Endocrinology and Infertility/Medical Genetics fellows, and interested graduate students (within the C.S. Mott Center) to become familiar with the PCR technique and how to use it effectively within the laboratory.

## **Teaching at Other Institutions**

#### Undergraduate Students

Adjunct Associate Professor. Taught two undergraduate courses, CHM104 "Introduction to Chemical Principles" and CHM201 "Introduction to Organic and Biological Chemistry" for nursing and health sciences students at the Department of Chemistry, Oakland University, Rochester, MI, 1991-2004.

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#### **Graduate Students**

Instructor. Four-day workshop: PCR Techniques, Concepts and Applications. Howard Hughes Research Program, Oakland University, Rochester, MI, May 19-22, 1998.

This workshop was for graduate, postdoctoral, laboratory research personnel, and faculty within the field of science and research.

Instructor. Taught a graduate course CHM554 "Molecular Biology and Biotechnology" at the Department of Chemistry, Oakland University, Rochester, MI, 1995-1998.

Instructor. Biotechnology: From Genes to Proteins. Department of Dermatology, Oakland University, Rochester, MI, 1993-1998.

This course was part of the Research Training in Biotechnology Program postgraduate curriculum for residents and fellows to utilize state-of-the-art molecular technology techniques to answer questions related to molecular pathogenesis of skin diseases such as skin cancer, fibrosis and wound healing.

Teaching Assistant. Introduction to Chemical Principals. Department of Chemistry, University of Essex, Colchester, England, United Kingdom, 1987-1988.

#### Residents and Fellows

Instructor and Laboratory Advisor. Biotechnology Research Training. Department of Dermatology, Oakland University, Rochester, MI, 1993-1998.

This program trained dermatology residents to utilize state-of-the-art molecular technology techniques to answer questions related to molecular pathogenesis of skin diseases such as skin cancer, fibrosis and wound healing.

#### Mentorship

Mentor on research projects related to endometriosis, postoperative adhesions, and ovarian cancer to the Department of Obstetrics and Gynecology past and present undergraduate and graduate students, residents, clinical and postgraduate fellows, scholars, faculty, and research technicians, assistants and associates.

#### Undergraduate Students:

Yousif Abbiss, Newaj Abdullah, Dana Abufarha, Shadi Abuolba Ahmad [awarded the 2007 Wayne State University School of Medicine Undergraduate Research Scholarship Award], Dabaja Ahmed, Ali Alarab, Radi Al-Dasouqi, Danna Al-Hadidi, Jeremy Berman, Chelsea Fortin, Ellory Greenberg, Waseem Imann, Shucni Jain, Marisa Karcz, Hadil Katato, Reem Khazaal, Yanamandra Krishnakant, Ira Memai, Wasfeh Musheinish, Bailey Neubauer, Osama Nusrat, Tessy Oommen, PageID: 230825

Norman Orabi, Alex Papadellis, Sonica Rehan, Mohammad Saed, James Waleke [WSU School of Medicine 2004 graduate], Rani Yaldo, Yousif Younan, Nabaa Zalzala, and Xuping (Sherry) Zhu.

#### Graduate:

<u>Batoul Abdullah, PhD</u>: \***Abdallah BY**, \*Horne SD, \*Stevens JB, \*Liu G, \*Ying AY, \*Vanderhyden B, Krawetz SK, Gorelick R, Heng HH (2013). Single cell heterogeneity: Why unstable genomes are incompatible with average profiles. Cell Cycle 12:3640-3649, 2013. PMID: 24091732 PMCID: PMC3903715

Role: Mentor/Advisor

<u>Jimmy Belotte, MD, PhD</u>: \*Belotte J, \*Fletcher NM, \*Saed MG, \*Abusamaan MS, \*Dyson G, Diamond MP, Saed GM. A single nucleotide polymorphism in catalase is strongly associated with ovarian cancer survival. PLoS One 10(8):e0135739, 2015. eCollection 2015. PMID: 26301412 PMCID: PMC4547699

<u>Amy Harper, MD</u>: \*Fletcher NM, \***Harper A**, \*Memaj I, \*Fan R, Morris RT, **Saed GM**. Molecular basis supporting the association of talcum powder use with increased risk of ovarian cancer. Reproductive Sciences. In press, 2019.

Nicole King, PhD: \*Fletcher NM, \*Belotte J, \*Saed MG, \*Memaj I, Diamond MP, Morris RT, Saed GM. Specific point mutations in key redox enzymes are associated with chemoresistance in epithelial ovarian cancer. Free Radical Biology and Medicine 102:122-132, 2017. PMID: 27890641

Osama Nusrat, MD, MS: \*Nusrat O, \*Belotte J, \*Fletcher NM, \*Memaj I, \*Saed MG, Diamond MP, Saed GM. The role of angiogenesis in the persistence of chemoresistance in epithelial ovarian cancer. Reproductive Sciences 23(11):1484-1492, 2016. PMID: 27122375

<u>Jennell White, PhD</u>: \*White JC, \*Jiang ZL, Diamond MP, Saed GM. Macrophages induce the adhesion phenotype in normal peritoneal fibroblasts. Fertility and Sterility 96(3):758-763.e3, 2011. Epub July 27, 2011. PMID: 21794857

#### Residents

Drs. (MD) Zeynep Alpay, Dana Ambler, Tarek Dbouk, Eslam Elhammady, and Valerie Shavell.

### Clinical Faculty and Clinical Postgraduate Fellows:

Drs. (MD) – Faculty: Awoniyi Awonuga, Associate Professor; Jimmy Belotte, Associate Professor; and Christopher Bryant, Associate Professor; Fellows: Mazen Abdallah, Jashoma Banerjee, Alan Bolnick, Jay Bolnick, Charalampos (Harry) Chatzicharalampous, Subodhsingh Chauhan, Laura Detti, Michael Freeman, April Gago, Amy Harper, Roohi Jeelani, Sana Khan, Mohamed Mitwally, Valerie Shavell, Mili Thakur, Rahi Victory, and Terri Woodard.

Of note, the aforementioned that have participated in premier scientific meetings such as: The American Society for Reproductive Medicine; American Gynecologic and Obstetrical Society; Society for Free Radical Biology and Medicine; Society for Reproductive Investigation; Society for Gynecologic Oncology; Pacific Coast Reproductive Society; Society for the Study of Reproduction; American Association for Cancer Research; Central American College of Obstetrics and Gynecology; and American College of Obstetrics and Gynecology, as well as publishing their many

scientific achievements (articles and abstracts) in the aforementioned preeminent, peer-reviewed journals (see Publications section, pg. 27). They have also participated and presented at local events: DMC/WSU Graduate Research Day; WSU Department of Obstetrics and Gynecology Resident and Fellow Day; WSU Obstetrics and Gynecology, CS Mott Center Reproductive Sciences Retreat; and the Michigan Alliance for Reproductive Technologies and Sciences (MARTS) Symposium.

### Acknowledgements:

Roohi Jeelani, MD [fellowship 2015-2017] and Sana Khan, MD [fellowship 2013-2016] of the Reproductive Endocrinology and Infertility Fellowship Program jointly received the Second Place Award for Outstanding Paper Presentation at the 65th Annual Meeting of the Pacific Coast Reproductive Society, Indian Wells, CA, in March of 2017. Their oral presentation was entitled, "Cyclophosphamide and Its Metabolite Impact on Fertilization through Mitochondrial Dysfunction."

Mili Thakur, MD [fellowship, 2014-2017] of the combined Reproductive Endocrinology and Infertility and Medical Genetics Fellowship Program received the First Place Award at the Department of Obstetrics and Gynecology Resident and Research Fellow Day, Wayne State University School of Medicine, Detroit, MI, in April of 2017. This award to Dr. Thakur was for her oral presentation entitled, "Galactose and Its Metabolites Deteriorate Metaphase II Mouse Oocyte Quality and Subsequent Embryo Development by Disturbing the Spindle Structure."

Mili Thakur, MD [fellowship, 2014-2017] of the combined Reproductive Endocrinology and Infertility and Medical Genetics Fellowship Program (the only one of its kind in the country), was the recipient of the 2016 Pfizer-SRI (Society for Reproductive Investigation), President's Presenter's Award. This award for given to Mili for her abstract entitled, "Galactose and Its Metabolites Deteriorate Metaphase II Mouse Oocyte Quality through a Mechanism that Involves the Generation of Reactive Oxidative Species, Mitochondrial Dysfunction and Apoptosis." The President's Presenter's Award is given in recognition of the 25 most meritorious abstracts (either poster or oral presentation) submitted by individuals still in training. Dr. Thakur received this prestigious award at the 63<sup>rd</sup> Annual Meeting of the Society for Reproductive Investigation, Montreal, Quebec, Canada, in March of 2016.

Alan Bolnick, MD and Sana Khan, MD [fellowship, 2013-2016] of the Reproductive Endocrinology and Infertility Fellowship Program were each awarded, from the Pacific Coast Reproductive Society, the 2015 Travel Award; as well as Roohi Jeelani, MD and Mili Thakur, MD [fellowship, 2015-2017 and 2014-2017, respectively] who were each awarded the 2016 Travel Award. These travel awards paid for registration to the annual meeting, course fees, and all travel expenses incurred.

<u>Michael Freeman, MD</u> [fellowship, 1999-2002] of the Reproductive Endocrinology and Infertility Fellowship Program was the recipient of a \$20,000 research grant from the American Gynecologic and Obstetrical Society (AGOS).

#### Scholars

<u>Iyad Ali, PhD</u>: Assistant Professor of Biochemistry, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine. Visiting Fulbright Arab Fund Fellowship Scholar [2013-2014] in the laboratories of Drs. Husam Abu-Soud and Ghassan Saed, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine. <u>Citation</u>: \*Maitra D,\* **Ali I**, \*Abdulridha RM, \*Shaeib F, \*Khan

SN, Saed GM, Pennathur S, Abu-Soud HM. PLoS One 9(11):e110595, 2014. eCollection 2014. PMID: 25375773

Awoniyi Awonuga, MD: Associate Professor, Women's Reproductive Health Research (WRHR) Scholar [2012-2015]. Division of Reproductive Endocrinology and Infertility. Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Citation: \*Awonuga AO, \*Belotte J, \*Abuanzeh S, \*Fletcher NM, Diamond MP, Saed GM. Advances in the pathogenesis of adhesion development: the role of oxidative stress. Reproductive Sciences 21(7):823-836, 2014. Epub February 11, 2014. Review. PMID: 24520085 PMCID: PMC4107571

Jimmy Belotte, MD, PhD: Associate Professor, Women's Reproductive Health Research (WRHR) Scholar [2012-2016], Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Citation: \*Belotte J, \*Fletcher NM, \*Alexis M, Morris RT, Munkarah AR, Diamond MP, Saed GM. Sox2 gene amplification significantly influences overall survival in serous epithelial ovarian cancer. Reproductive Sciences 22(1):38-46, 2015. Epub July 18, 2014. PMID: 25038052 PMCID: PMC4275450

Lylia Fahmy, MD: Clinical Instructor, Women's Reproductive Health Research (WRHR) Scholar [2001-2003], Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Thesis: Effect of Ovarian Hormones on Adhesion Development.

### Faculty

Mentor of current and past Obstetrics and Gynecology clinical faculty through collaborations on research projects and grant submissions. Faculty members are as follows: Awoniyi Awonuga, MD, Professor, Division of Reproductive Endocrinology and Infertility; Jimmy Belotte, MD, PhD, Associate Professor [past], Division of Gynecology; Christopher Bryant, MD, Associate Professor [past], Division of Gynecology; Lylia Fahmy, MD, Clinical Instructor [past], Division of Reproductive Endocrinology and Infertility; and Peter Baumann, MD, Associate Professor [retired], Division of Gynecology.

I have also been instrumental to key professional presentations at local, national, and international conferences by our past and present senior faculty members of the Obstetrics and Gynecology Department. They are as follows: Jay Berman, MD, Associate Professor and Associate Chair, Director, Division of Gynecology; Michael Diamond, MD, Professor and Associate Chair [past], Director, Division of Reproductive Endocrinology and Infertility [past], and Assistant Dean of Clinical and Translational Research [past, now at Georgia Regents University]; Bernard Gonik, MD, Professor, Division of Maternal and Fetal Medicine; John Malone, Jr, MD, Professor and Chair [past, deceased]; Kamran Moghissi, MD, Professor Emeritus and Chair Emeritus [past], Director, Division of Reproductive Endocrinology and Infertility [past], and Director, CS Mott Center for Human Growth and Development [past, retired]; and Adnan Munkarah, MD, Professor and Director, Division of Gynecologic Oncology.

#### Research Associates/Assistants/Technicians

In the laboratory of Dr. Ghassan Saed: Drs. (PhD) Boytcho Boytchev, Nicole King, Semira Galijasevic, Zhongliang (John) Jiang, MD, Hong Lu, Qui Lu, Gheorghe Proteasa, Natalie Rizk, Rona Wang, MD, and Ming Zhao, MD; Danielle Hall, BS, Ira Memaj, BS, and Manal Omar, BS.

## **Essays/Theses/Dissertations Directed**

Osama Nusrat, MD, MS, Department of Physiology in the Reproductive Sciences (2015-2017), Wayne State University School of Medicine, Detroit, MI

Document 33111-33

PageID: 230828

Dissertation Title: The Role of Angiogenesis in the Persistence of Chemoresistance in Epithelial Ovarian Cancer

Date Awarded: Master degree, September 2017

Current Status: Resident, Department of Internal Medicine, University of Arizona College of Medicine, Tucson, AZ

Jimmy Belotte, MD, PhD, Associate Professor, Department of Physiology in the Reproductive Sciences Concentration (2012-2016); and Women's Reproductive Health Research (WRHR) Scholar, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit. MI

Dissertation Title: The Role of Oxidative Stress in the Establishment of Resistance to Cisplatin in **Epithelial Ovarian Cancer Cells** 

Date Awarded: PhD Degree, Sept. 14, 2016 and WRHR training completed Sept. 2016

Current Status: Associate Professor, Department of Obstetrics and Gynecology, Division of Gynecology, Montefiore Medical Center, Bronx, NY

Batoul Abdullah, PhD. Department of Physiology in the Center for Molecular Medicine and Genetics Concentration (2012-2016), Wayne State University School of Medicine, Detroit, MI Dissertation Title: Fuzzy Inheritance: A Novel Form of Somatic Cell Inheritance that Regulates Cell Population Heterogeneity.

Date Awarded: PhD Degree, 2016

Current Status: Postdoctoral Fellow in the laboratory of Henry (Hong-Qiang) Heng, PhD, Center for Molecular Medicine & Genetics and Pathology, Wayne State University School of Medicine, Detroit. MI

Awoniyi Awonuga, MD, Women's Reproductive Health Research (WRHR) Scholar [2012] 2015], Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Detroit. MI

Thesis Title: Oxidative Stress in the Pathogenesis of Post-Operative Adhesions

Training Completed: December 2015

Current Status: Professor and Interim Director, Division of Reproductive Endocrinology and Infertility and Director, Residency Program Research, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI

Nicole King, PhD, Department of Physiology in the Reproductive Sciences Concentration Program (2008-2013), Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Detroit. MI

Dissertation Title: The Role of Oxidative Stress in the Pathogenesis of Epithelial Ovarian Cancer Date Awarded: PhD Degree, August 2013

Current Status: Postdoctoral Fellow in the laboratory of Ghassan M Saed, PhD, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, CS Mott Center for Human Growth and Development, Wayne State University School of Medicine, Detroit, MΙ

Jennell White Jackson, PhD, Department of Physiology in the Reproductive Sciences Concentration Program (2000-2011), Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI

PageID: 230829

Dissertation Title: The Potential Role of Innate Immunity in the Pathogenesis of Postoperative Adhesions

Date Awarded: PhD Degree, September 2011

Current Status: Postdoctoral Fellow, Department of Pediatrics, Wayne State University School of

Medicine, Detroit, MI

Lylia Fahmy, MD, Women's Reproductive Health Research (WRHR) Scholar [2001-2003], Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MΙ

Thesis Title: Effect of Ovarian Hormones on Adhesion Development

Training Completed: September 2003

Current Status: Associate Professor, Department of Obstetrics and Gynecology, University of

Nebraska Medical Center, Omaha, NB

#### **Course or Curriculum Development**

Originator and Director. Summer Reproductive Technology Course. 2014 This course design is to allow for Reproductive Endocrinology and Infertility/Medical Genetics fellows, as well as graduate students, to become familiar with all aspects of laboratory techniques within the field of reproductive sciences. Division of Reproductive Endocrinology and Infertility. Department of Obstetrics and Gynecology, The C.S. Mott Center for Human Growth and Development, Wayne State University School of Medicine, Detroit, MI.

Course Director. Reproductive Sciences Concentration – RPS 7350: 2006 Biomolecular Techniques: From Genes to Protein.

This course design is specifically for graduate students enrolled in The PhD Program in Physiology with Concentration in the Reproductive Sciences, as part of their curriculum. This is an integrated PhD program incorporating the teaching, research, and physical resources of two departments -- Physiology and Obstetrics & Gynecology at Wayne State University School of Medicine, Detroit, MI.

Organizer. Four-day workshop (May 19-22): PCR Techniques, Concepts, 1998 and Applications.

Workshop developed for undergraduates, graduates, postdoctoral, laboratory personnel, and faculty studying and/or working within the field of science and research. Sponsored by the Howard Hughes Research Program of Oakland University, Rochester, MI.

Designer. Introduction to Molecular Cloning. 1996

Course designed to teach techniques for characterization and manipulation of DNA and RNA from the basis of modern biomedical research. Coursework pertinent towards medical residents and fellows at the Henry Ford Hospital. Detroit, MI, and graduate students at Oakland University, Rochester, MI.

Designer. Research Training in Biotechnology. 1993 This program trained Department of Dermatology residents and fellows to utilize state-of-the-art molecular technology techniques to answer questions related to molecular pathogenesis of skin diseases such as skin cancer, fibrosis and wound healing at Henry Ford Hospital, Detroit. MI. This training ended in 1998.

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Course Director. I have participated in developing the course, Introduction to Chemical Principles (CHM 104) to meet general education requirements. CHM 104 satisfies the university general education requirement in natural science and technology (NST). The learning outcomes for NST courses state that the student will demonstrate knowledge of major concepts from natural science or technology, including developing and testing of hypotheses, drawing conclusions, and reporting of findings through some laboratory experience or an effective substitute. This course taught at Oakland University, Rochester, MI.

1991-2004

Designer. Laboratory course. I was actively involved in developing and instructing two laboratory sections for CHM 104. Students learned how to evaluate sources of information in science or technology. Developed at Oakland University, Rochester, MI

1991-2004

<u>Designer</u>. I developed and taught CHM 104 and CHM 201 to nursing students on-line (a web-based instruction). I designed courses to satisfy the university general education requirement in natural science and technology (NST). For this, I utilized and implemented the virtual chemistry laboratory experience to be an integral part of this course. Developed at Oakland University, Rochester, MI,

2005-2010

### **GRANTS, CONTRACTS, AND OTHER FUNDING:**

#### **Active National/International Grants and Contracts**

Role: Principal Investigator, Percent Effort: 10% Title: Novel target for ovarian cancer treatment

Source: DS Biotech LLC. Date: 01/01/2021- 12/31/2021 Total Direct Costs: \$100,000

# **Pending National/International Grants and Contracts**

None

#### **Submitted National/International Grants and Contracts**

Role: Principal Investigator, Percent Effort: 30%

Title: Monomeric MPO as a biomarker for early detection of ovarian cancer

Source: NIH/NICHD R01 Date: 09/01/2020 - 08/31/2025 Total Direct Costs: \$1,250,000

Role: Principal Investigator, Percent Effort: 30% Title: Novel mechanism of survival in EOC cells

Source: NIH/NICHD R21

Date: 09/01/2020 – 09/01/2022 Total Direct Costs: \$375,000

Role: Principal Investigator, Percent Effort: 30%

Title: Binding of intracellular monomeric MPO to aV/b1 integrin serves as a novel mechanism of

survival in EOC cells. Source: NIH/NICHD R01

Date: 09/01/2019 – 08/31/2024 Total Direct Costs: \$1,250,000

Role: Principal Investigator, Percent Effort: 10%

Title: Binding of intracellular monomeric MPO to aV/b1 integrin serves as a novel mechanism of

survival in EOC cells.

Source: DOD

Date: 01/01/2018 – 01/01/219 Total Direct Costs: \$385,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Mechanism of Apoptosis in Chemoresistant Ovarian Cancer Cells." To determine whether chenoresistance in ovarian cancer manifests decreased apoptosis through enhanced snitrosylation of caspase-3 mechanism which can, thereby, be reversed by DCA.

Source: American Association for Cancer Research (AACR)

Date: 07/01/17 – 06/30/19 Total Direct Costs: \$100,000

Role: Principal Investigator, Percent Effort: 30%

Title: "Identification of a Novel Target with Intriguing Anti-Tumorigenic Effects in Ovarian Cancer." To identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.

Source: NIH/NICHD R01, Proposal #17-0220

Date: 09/01/17 – 08/31/22 Total Direct Costs: \$1,919,600

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Marker of Survival in Ovarian Cancer Cells." To test the anti-tumorigenic potential of integrin  $\alpha V/\beta 1$  antibodies in sensitive and chemoresistant ovarian cancer.

Source: U.S. Department of Defense (DOD)

Date: 01/01/18 – 12/31/19 Total Direct Costs: \$385,000

Role: Principal Investigator, Percent Effort: 25%

Title: "Cross-Talk Between MPO and iNOS Regulates Apoptosis in Chemoresistant Ovarian Cancer." To determine whether chemoresistance in ovarian cancer manifests decreased apoptosis through enhanced s-nitrosylation of caspase-3 mechanism, which can be reversed by DCA.

Source: NIH/NICHD R21 Date: 09/01/17 – 08/31/19 Total Direct Costs: \$423,500

Role: Principal Investigator, Percent Effort: 10%

Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Cancer." To identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.

Source: NIH/DHHS Small Business Technology Transfer Grant (STTR), R41

Date: 07/01/17 - 6/30/18 Total Direct Costs: \$299,999

Role: Principal Investigator, Percent Effort: NA

Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Cancer." To identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.

Source: The Honorable Tina Brozman Foundation, Inc. for Ovarian Cancer Research – Letter of

Intent

Date: 2017

Total Direct Costs: \$100,000

Role: Principal Investigator, Percent Effort: 5%; Co-Principal Investigator: NM King, PhD

Title: "Potential Anti-Tumorigenic Antigen for Cancer Therapy." To identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.

Source: Elsa U. Pardee Foundation Grant Program, Proposal #17-0715

Date: 01/01/18 - 12/31/18 Total Direct Costs: \$187,958

Role: Principal Investigator, Percent Effort: NA

Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Cancer." To identify and test a target that cross-reacts with the CD11b antibody and determines it efficacy in killing both sensitive and chemoresistant ovarian cancer cells.

Source: Ovarian Cancer Research Fund Alliance, Inc. (OCRFA)

Date: 01/01/18 - 12/31/20 Total Direct Costs: \$300,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Antitumor Effects of Targeting Integrin αV/β1 in Ovarian Cancer Cells." To test the anti-

tumorigenic potential of integrin αV/β1 antibodies in ovarian cancer patient samples.

Source: Ovarian Cancer Research Fund Alliance, Inc., Ann Schreiber Mentored Investigator

Award

Date: 01/01/18 - 12/31/18 Total Direct Costs: \$75,000

#### **Submitted Other Grants and Contracts**

Role: Principal Investigator

Title: "Repurposing ABCIXIMAB, A Clinically Approved Anticoagulant for the Treatment of Ovarian Cancer." To determine whether abciximab is an effective therapy against sensitive and resistant ovarian cancer.

Source: Michigan Ovarian Cancer Alliance (MIOCA)

Date: 04/01/17 - 03/31/18 Total Direct Costs: \$50,000

Role: Principal Investigator

Title: "ReoPro and Ovarian Cancer"

Source: Michigan Ovarian Cancer Alliance (MIOCA)

Date: 04/01/17 - 03/31/18 Total Direct Costs: \$50,000

Role: Principal Investigator

Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Cancer"

Source: DS Biotech, LLC, Proposal #17-0289

Date: 07/01/17-06/30/18 Total Direct Costs: \$100,000

Role: Principal Investigator

Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Ovarian Cancer"

Source: Rivkin Center for Ovarian Cancer, Pilot Study Awards, 573569

## **Previously Funded Grants and Contracts**

Role: Principal Investigator, Percent Effort: 5%

Title: "Elucidation of Cellular Mechanisms of Evitar of Post-Operative Fibrosis."

Source: Temple Therapeutics, 25S8P1

Date: 04/01/17 - 05/31/18 Total Direct Costs: \$100.000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The project's design is to identify key markers of oxidative stress that have the potential to serve as screening tools for ovarian cancer and may play a role in the acquisition of chemoresistance.

Source: Prevent Cancer Foundation, Postdoctoral Fellowship Grant

Date: 01/01/16 - 1/31/18 Total Direct Costs: \$80,000

Role: Co-Principal Investigator; Principal Investigators: MP Diamond, MD, EN Kraiselburd, PhD Title: "WSU-UPR Research Partnership to Promote Diversity in the Reproductive Sciences"

Source: NIH/NICHD, HD-09-008

Date: 08/2010 - 07/2015 Total Direct Costs: \$3,020,000

Role: Co-Principal Investigator, Principal Investigator: MP Diamond, MD Title: "WSU Clinical and Translational Science Award Planning Grant"

Source: NIH/NICHD, 1P20 RR 023578

Date: 09/2006 - 09/2012 Total Direct Costs: \$2,225,750

Role: Consultant; Principal Investigator: MP Diamond, MD Title: "WSU Cooperative Reproductive Medicine Network Center"

Source: NIH/NICHD, U10 HD-39005

Date: 08/2007 - 07/2012 Total Direct Costs: \$1,510,000

Role: Principal Investigator, Percent Effort: 3.60%

Title: "Postoperative Adhesion: Roles of Hypoxia and Nitric Oxide"

Source: NIH/NICHD, Division of Pharmacology, Physiology, and Biological Chemistry, 1R01

GM069941-01A3 Date: 10/01/06 – 09/30/12 Total Direct Costs: \$1,312,500

Role: Mentor; Principal Investigator: J White, MS, PhD Candidate (WSU) Title: "Post-Operative Adhesions: Roles of Hypoxia in Nitric Oxide"

Source: NIH/NICHD, Minority Research Supplemental Award, 3R01GM069941-02S1

Date: 01/01/08 – 08/31/10 Total Direct Costs: \$151,441

Role: \*Principal Investigator

Title: "\*CUAAH Subcontract - Specialty Laboratory Core"

Date: 06/01/08 – 05/31/10 Total Direct Costs: \$403,840

Role: Co-Principal Investigator; Principal Investigator: JM Flack, MD

Title: "Center for Urban African American Health (CUAAH)"

Source: NIH/NIEHS Date: 06/01/07 – 05/31/10

Total Direct Costs for Center: \$9,487,709

Role: Principal Investigator

Title: "Angiogenesis of Ovarian Cancer"

Source: Frank lacobell Endowed Chair, Department of Obstetrics and Gynecology, Wayne State

University School of Medicine Date: 01/01/08 – 12/31/09 Total Direct Costs: \$41,500

Role: Co-Principal Investigator; Principal Investigator: R Kannan, PhD Title: "Wayne State University, Department of Engineering – Subcontract" Source: President's Research Award, Technology and Transfer Office

Date: 01/01/08 – 12/31/09 Total Direct Costs: \$15,000

Role: Consultant; Principal Investigator: MP Diamond, MD U10 HD-39005

Title: "WSU Cooperative Reproductive Medicine Network Center"

Source: NIH/NICHD Date: 04/01/00 – 03/31/07 Total Direct Costs: \$1,349,994

Role: Principal Investigator; Co-Principal Investigator: MP Diamond, MD

Title: "Testing of Perfluorodecalin for Adhesion Prevention"

Source: Novel Pharma, Inc. Date: 11/01/01 – 06/30/02 Total Direct Costs: \$32,000

Role: Principal Investigator; Co-Principal Investigator: MP Diamond, MD

Title: "Effect of Tissel on Human Peritoneal Fibroblasts"

Source: Baxter Research Grant

Date: 09/30/01 – 12/31/02 Total Direct Costs: \$98,000

Role: Co-Principal Investigator; Principal Investigator: MP Diamond, MD

Title: "Why Does Endometriosis Cause Adhesions?"

Source: Endometriosis Association

Date: 01/01/01 – 12/31/01 Total Direct Costs: \$38,000

Role: Co-Principal Investigator; Principal Investigator: MP Diamond, MD

Title: "Effect of Tissel on Human Mesothelial Cell Culture"

Source: Baxter Research Grant Date: 01/01/01 – 08/31/01 Total Direct Costs: \$60,000

Role: Principal Investigator

Title: "The Effects of Hypoxia on the Levels of Peritoneal ECM Proteins"

Source: Wayne State University Department of Obstetrics and Gynecology, Interdepartmental

Research Grant

Date: 03/01/98 – 12/31/00 Total Direct Costs: \$19,000

Role: Principal Investigator

Title: "The Role of p53 in the Pathogenesis of Keloids" Source: Henry Ford Hospital Small Project Award

Date: 01/01/98 – 12/31/98 Total Direct Costs: \$20,000

Role: Principal Investigator

Title: "Patterns of Cytokine Expression in Cutaneous T-Cell Lymphoma"

Source: Henry Ford Hospital Small Project Award

Date: 01/01/93 – 12/31/94 Total Direct Costs: \$20,000

#### **Previously Submitted, Not Funded Grants and Contracts**

Role: Principal Investigator, Percent Effort: 20%

Title: "Novel Mechanisms of Apoptosis in Chemoresistant Ovarian Cancer Cells." To determine whether chenoresistance in ovarian cancer manifests decreased apoptosis through enchanged snitrosylation of caspase-3 mechanism, which can be reversed by DCA.

Source: NIH/NICHD, R21 Date: 04/01/17 – 03/31/19 Total Direct Costs: \$423,500

Role: Principal Investigator

Title: "Novel Mechanisms of Apoptosis in Chemoresistant Ovarian Cancer Cells." To determine whether chenoresistance in ovarian cancer manifests decreased apoptosis through enchanged snitrosylation of caspase-3 mechanism, which can be reversed by DCA.

Source: Elsa U. Pardee Foundation Grant Program

Date: 01/01/17 – 12/31/17 Total Direct Costs: \$113,966 Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Mechanisms of Apoptosis in Chemoresistant Ovarian Cancer Cells." The project's design is to determine whether chemoresistance in ovarian cancer manifests decreased apoptosis through enhanced s-nitrosylation of caspase-3 mechanism, which can be reversed by DCA.

Source: NIH/NICHD, R03 Date: 12/01/16 - 11/30/18 Total Direct Costs: \$50,000

Role: Principal Investigator

Title: "Innovative New Target for Ovarian Cancer Therapy." The project was designed to identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.

Source: The Honorable Tina Brozman Foundation, Inc. for Ovarian Cancer Research

Date: 08/01/16 - 07/31/18 Total Direct Costs: \$200,000

Role: Principal Investigator

Title: "Redox Enzyme-Mediated Prosurvival of Chemoresistance in Ovarian Cancer." To determine whether development of chenoresistance in ovarian cancer is attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.

Source: Ovarian Cancer Research Fund Alliance, Inc. (OCRFA)

Date: 2016 – 2019

Total Direct Costs: \$900,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Chemoresistant Ovarian Cancer Cells Manifest Lower Vascular Endothelial Growth Factor and Hypoxia Inducible Factor-1α: A Potential Survival Mechanism." The design of the project was to determine whether VEGF and HIF-1 $\alpha$  contribute to the persistence of chemoresistance in ovarian cancer.

Source: Ovarian Cancer Research Fund, Ann Schreiber Mentored Investigator Award

Date: 2016 – 2017

Total Direct Costs: \$75,000

Role: Mentor, Percent Effort: 0%; PI: NM King, PhD

Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

Source: NIH/NICHD, R03 – Resubmission of scored proposal

Date: 12/01/15-11/30/17 Total Direct Costs: \$153,583

Role: Principal Investigator, Percent Effort: 30%

Title: "Redox Enzyme-Mediated Prosurvival of Chemoresistance in Ovarian Cancers." The design of the project was to determine whether development of chemoresistance in ovarian cancer is attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.

Source: NIH/NICHD, R01 Date: 12/01/15 - 11/30/20 Total Direct Costs: \$2,494,526

Role: Mentor, Percent Effort: 0%; PI: NM King, PhD

Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

Source: NIH/NICHD, R03 Date: 11/01/14 – 10/31/16 Total Direct Costs: \$152,000

Role: Principal Investigator, Percent Effort: 30%

Title: "Chemoresistance Induces a Genotype Switch in Redox Enzymes in Ovarian Cancer." The design of the project was to determine whether development of chemoresistance in ovarian cancer is attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.

Source: NIH/NICHD, R01 Date: 04/ 01/15 – 03/31/20 Total Direct Costs: \$3,124,495

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

Source: Sandy Rollman Ovarian Cancer Foundation (SROCF) Fellowship

Date: 06/01/14 – 05/31/15 Total Direct Costs: \$50,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

Source: Ladies Auxiliary to the Veterans of Foreign Wars, Postdoctoral Cancer Research

Fellowship

Date: 06/01/14 – 05/31/16 Total Direct Costs: \$50,000

Role: Mentor, Percent Effort: 0%; PI: NM King, PhD

Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

Source: Kaleidoscope of Hope Foundation, Young Investigator Award

Date: 04/01/14 – 03/31/15 Total Direct Costs: \$50,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

Source: Damon Runyon Cancer Research Foundation

Date: 07/01/14 – 06/30/17 Total Direct Costs: \$158,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Chemoresistance Induces a Genotype Switch in Epithelial Ovarian Cancer Cells." The project was designed to determine whether development of chemoresistance in ovarian is attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.

Source: American Cancer Society Postdoctoral Fellowship

Date: 01/01/15 - 12/31/18 Total Direct Costs: \$163,500

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Biomarkers for the Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

Source: American Association for Cancer Research

Date: 2015 – 2016

Total Direct Costs: \$50,000

Role: Principal Investigator, Percent Effort: 30%

Title: "Postoperative Adhesion Development is Controlled by Mechanisms that Emanate from a Hypoxia-Induced Genotype Switch in Key Enzymes of Oxidative Stress." Identification of markers that are strongly associated with adhesions and in patients will contribute to both the delineation of mechanisms of adhesion development and serve as potential targets for therapy and intervention.

Source: NIH/NICHD, R01 Date: 07/01/15 - 06/30/20 Total Direct Costs: \$1,921,633

Role: Principal Investigator, Percent Effort: 25%

Title: "Combination of Antioxidants Effectively Reduces Adhesion Development." The design of the project was to determine the effects of antioxidants on the prevention of postoperative adhesion development.

Source: NIH/NICHD, R03 Date: 07/01/15 - 06/30/17 Total Direct Costs: \$153,314

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "New Insights into the Pathogenesis of Ovarian Cancer." To identify keymarkers of oxidative stress that have the potential to serve as screening tools for ovarian cancer and may play a role in the acquisition of chemoresistance.

Source: Prevent Cancer Foundation

Date: 04/01/14 - 01/31/16 Total Direct Costs: \$80,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: AO Awonuga, MD

Title: "Effects of Dietary Lycopene on Incidence and Severity of Postoperative Adhesions." The design of the project was to determine the effects of antioxidants on the prevention of postoperative adhesion development.

Source: NIH/NICHD, R03 Date: 09/01/14 - 08/31/16 Total Direct Costs: \$152,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Biomarkers for Detection of Early Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

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Source: Marsha Rivkin Center for Ovarian Cancer Research, Scientific Scholar Award

Postdoctoral Fellowship Date: 04/01/14 - 03/31/15 Total Direct Costs: \$60,000

Role: Mentor; Percent Effort: 0%; Principal Investigator: J Belotte, MD

Title: "Catalase SNP as a Genetic Predictor for Epithelial Ovarian Cancer." The design of the project was to determine whether a SNP in the catalase gene could be utilized as a predictive marker for epithelial ovarian cancer.

Source: Marsha Rivkin Center for Ovarian Cancer Research, Scientific Scholar Award

Postdoctoral Fellowship Date: 04/01/14 - 03/31/15 Total Direct Costs: \$60,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Biomarkers for the Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.

Source: Hope Funds Cancer Research Postdoctoral Fellowship

Date: 2014 – 2016

Total Direct Costs: \$100,000

Role: Principal Investigator

Title: "Chemoresistance in Ovarian Cancer Manifests a Genotype Switch in Oxidant Enzymes." The design of the project was designed to determine whether a genotype switch in key oxidant enzymes is induced in chemotherapy treated ovarian cancer cells and the subsequent effect of the enzymatic activity.

Source: Marsha Rivkin Center for Ovarian Cancer Research; Pilot Study

Date: 04/01/14 - 03/31/15 Total Direct Costs: \$75,000

Role: Principal Investigator, Percent Effort: 30%; Co-Investigators: MP Diamond, MD, S

Ghamande, PhD

Title: "Chemoresistance Induces a Genotype Switch in Redox Enzymes in Ovarian Cancer." The design of the project was to determine whether development of chemoresistance in ovarian cancer were attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.

Source: NIH/NICHD, R01 Date: 07/01/14 - 06/30/19 Total Direct Costs: \$2,932,687

Role: Principal Investigator, Percent Effort: 25%

Title: "Chemoresistance in Ovarian Cancer is attributed to Enhanced Oxidative Stress." The design of the project was to determine whether development of chemoresistance in ovarian cancer were attributed to enhanced oxidative stress.

Source: NIH/NICHD, R03 Date: 07/01/14 - 06/30/16 Total Direct Costs: \$152,000 PageID: 230840

Role: Principal Investigator, Percent Effort: 25%

Title: "Chemoresistance in Ovarian Cancer Manifests a Genotype Switch in Oxidant Enzymes." The design of the project was to determine whether development of chemoresistance in ovarian cancer were attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.

Source: NIH/NICHD, R03 Date: 07/01/14 - 06/30/16 Total Direct Costs: \$152,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: J Belotte, MD

Title: "Characterization of Epithelial Ovarian Cancer Stem Cells." The design of the project was to determine the role of pluripotency markers in epithelial ovarian cancer and the association with survival.

Source: NIH/NICHD, R03 Date: 09/30/14 - 06/30/16 Total Direct Costs: \$152,000

Role: Mentor, Percent Effort: 0%; PI: J Belotte, MD

Title: "Catalase SNP as a Genetic Predictor for Epithelial Ovarian Cancer." The design of the project was to determine whether a SNP in the catalase gene could be utilized as a predictive marker for epithelial ovarian cancer.

Source: NIH/NICHD, R03 Date: 09/30/14 - 08/31/16 Total Direct Costs: \$152,000

Role: Principal Investigator, Percent Effort: 20%

Title: "Innovative New Target for Ovarian Cancer Therapy." The project was designed to identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.

Source: NIH/NICHD, R21 Date: 12/31/16 - 11/30/18 Total Direct Costs: \$275,000

Role: Principal Investigator

Title: "Redox Enzyme-Mediated Prosurvival of Chemoresistance in Ovarian Cancer." The design of the project was to determine whether development of chemoresistance in ovarian cancer were attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.

Source: Ovarian Cancer Research Fund, Program Project Development Grant

Date: 201 6 - 2019

Total Direct Costs: \$900,000

Role: Principal Investigator, Percent Effort: 20%

Title: "Novel Mechanisms of Apoptosis in Chemoresistant Ovarian Cancer Cells." The design of the project was to determine whether chemoresistance in ovarian cancer manifests decreased apoptosis through enhanced s-nitrosylation of caspase-3 mechanism, which could be reversed by DCA.

Source: NIH/NICHD, R21 Date: 12/01/16 - 11/30/18 Total Direct Costs: \$275,000 PageID: 230841

Role: Mentor, Percent Effort 0%; Principal Investigator: NM King, PhD

Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron can be utilized as biomarkers for the early detection of ovarian cancer.

Source: L'Oreal USA for Women in Science Fellowship

Date: 2016 – 2017

Total Direct Costs: \$60,000

#### PATENTS:

#### COMPOSITIONS AND METHODS TO TREAT SOLID TUMORS

Publication Number: 20190309073

Abstract: Compositions and methods that utilize anti-CD11b antibodies, anti-CD18 antibodies, anti-myeloperoxidase (MPO) antibodies, anti-integrin alpha-V antibodies, anti-integrin beta-1 antibodies, Abciximab, neutrophil inhibitory factor (NIF) protein, and/or combinations thereof to treat solid tumor cancers are described.

Type: Application Filed: May 20, 2019

Publication Date: October 10, 2019 Applicant: Wayne State University Inventor: Ghassan M. Saed

# METHOD OF PREVENTING ADHESIONS BY APOPTOSIS OF ADHESION PERITONEAL

CELLS

Publication Number: 20040131600

Abstract: Methods for the prevention of adhesion formation and development involve the administration of therapeutic formulations to a patient which modulates the rate of apoptosis of adhesion fibroblast cells. The formulations preferably include Bax, Bax enhancers, such as p53, Bax agonists, Bcl-2 inhibitors and Bcl-2 antagonists. A method is also provided for determining the predisposition of a subject to adhesion formation by measuring the Bcl-2/Bax ratio at multiple sites within the subject.

Type: Application Filed: March 1, 2004

Publication Date: July 8, 2004 Inventor: Ghassan M. Saed

#### MODEL FOR IN VITRO ADHESION DEVELOPMENT

Publication Number: 20040096817

Abstract: A biological model for the development of adhesions in vitro comprises a pair of opposed surfaces of tissue explants maintained in a culture media for a sufficient time and under conditions to permit the formation of adhesions. The model is useful for evaluating compounds and techniques for the prevention and remediation of adhesions, and for individualizing the therapeutic options for patients who may experience adhesions.

Type: Application Filed: May 28, 2003

Publication Date: May 20, 2004 Applicant: Wayne State University Inventor: Ghassan M. Saed

#### METHODS OF TREATING CANCER WITH CD 11B ANTIBODIES

International Publication Number: WO 2010/017083 AI International Publication Date: February 11, 2010

Description of Patent: Methods and compositions for treating cancer with CDI lb antibodies are disclosed. The antibodies may be WT.5 antibodies or compete with WT.5 antibodies, and induce

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apoptosis in SKOV, MDAH-2274, or BxPC-3 cells.

Inventor: Ghassan M. Saed

#### METHOD OF PREVENTING ADHESIONS WITH INTERFERON-GAMMA

International Publication Number: WO 02/072016 A3 International Publication Date: September 19, 2002

Description of Patent: Methods for the prevention of adhesion formation and development involve the administration of therapeutic formulations to a patient which include, as active ingredients, IFNy or IFN-y enhancers. The IFN-y or IFN-y enhancers are preferably administered to fibrosis tissues in a subject prior to an event which induces adhesion formation, such as a surgical event.

Inventor: Ghassan M. Saed

## METHODS FOR THE PREVENTION OF ADHESION FORMATION AND DEVELOPMENT

Patent Number: US2006025364 Application Number: US20050081278 Application Date: March 16, 2005 Publication Date: February 2, 2006

Description of Patent: Methods for the prevention of adhesion formation and development, and for the stimulation of fibrosis, involve the administration of therapeutic formulations to a patient containing inhibitors or stimulators to selected molecular adhesion markers. The molecular markers of the invention include Caspase 2, Caspase 3, Caspase 9, PPAR alpha, PPAR beta,

PPAR gamma1, PPAR gamma2, and NF-kappa B.

Inventor: Ghassan M. Saed

#### **PUBLICATIONS**

## **Peer-Reviewed Publications**

\*Indicates student, trainee, or postdoctoral

### **Reports of Original Work**

1. Awonuga A.O., Chatzicharalampous C., Thakur M., Rambhatla R., Qadri F., Awonuga M., Saed GM., Diamond MP. Genetic and Epidemiological Similarities, and Differences Between Postoperative Intraperitoneal Adhesion Development and Other Benign Fibroproliferative Disorders. Reprod. Sci. (2021). https://doi.org/10.1007/s43032-021-00726-9

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- 2. Thakur M., Rambhatla A., Qadri F., Chatzicharalampous C., Awonuga M., Saed GM., Diamond MP., Awonuga, A.O. Is There a Genetic Predisposition to Postoperative Adhesion Development? Reprod. Sci. 28, 2076-2086 (2021). https://doi.org/10.1007/s43032-020-00356-7
- Irene Peregrin-Alvarez, Nicole M Fletcher, Ghassan M Saed, Robert A Roman, Laura Detti. Anti-Müllerian Hormone (AMH) regulates BRCA1 and BRCA2 gene expression after ovarian cortex transplantation. Gynecol Endocrinol. 2020 Oct 21:1-4. PMID: 33084436
- 4. Laura Detti, Mustafa I Abuzeid, Irene Peregrin-Alvarez, Mary E Christiansen, Pouran Malekzadeh, Jennifer Sledge, Ghassan M Saed. Recombinant Anti-Müllerian Hormone (rAMH) for Stalling In Vitro Granulosa Cell Replication. Reprod. Sci. 2020 July2; (27) 1873-1878. Doi: 10.1007/s43032-020-00206-6
- 5. \*Harper A, \*Fletcher NM, \*Fan R, Morris RT, **Saed GM.** Heat Shock Protein 60 (HSP60) Serves as A Potential Target for the Sensitization of Chemoresistant Ovarian Cancer Cells. Reprod Sci. 2020 Apr27;(4):1030-1036. doi: 10.1007/s43032-019-00089-2. Epub 2020 Mar 2.PMID: 32124395
- 6. \*Fletcher NM, \*Harper A, \*Memaj I, \*Fan R, Morris RT, Saed GM. Molecular basis supporting the association of talcum powder use with increased risk of ovarian cancer. Reproductive Sciences. 2019 Dec; 26(12) P1603-1612
- 7. Detti L. \*Fletcher NM. Uhlmann RA. Peregrin-Alvarex I. Roman RA. Saed GM. Anti-Müüllerian hormone (AMH) regulates stemness-promoting factors in fresh and previously vitrified-warmed ovarian cortex. Minverva Ginecologica. 2019 Feb 6. doi: 10.234736/S0026-4784.19.04276-X. [Epub ahead of print] PMID: 30727722
- 8. Saed GM, \*Fletcher NM, Diamond MP, Morris RT, Gomez-Lopez N, \*Memaj I. Novel expression of CD11b in epithelial ovarian cancer: Potential therapeutic target. Gynecologic Oncology. 2018 Mar;148(3):567-575. doi: 10.1016/j.ygyno.2017.12.018. Epub 2018 Jan 10. PMID: 29329880
- 9. Robertson LM, \*Fletcher NM, Diamond MP, Saed GM. Evitar (I-Alanyl-I-Glutamine) regulates key signaling molecules in the pathogenesis of postoperative tissue fibrosis. Reproductive Sciences. 2018 Sep 5:1933719118789511. 10.1177/ 1933719118789511. [Epub ahead of print] PMID: 30185141

10. Detti L, \*Fletcher NM, Saed GM, Sweatman TW, Uhlmann RA, Pappo A, Peregrin-Alvarez I. Xenotransplantation of pre-pubertal ovarian cortex and prevention of follicle depletion with anti-Müllerian hormone (AMH). Journal of Assisted Reproduction and Genetics 35(10):1831-1841, 2018. doi: 10.1007/s10815-018-1260-z. Epub July 25, 2018. PMID: 30043336

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Role: Mentor and collaborator

- 11. Detti L, \*Fletcher NM, Saed GM, Peregrin-Alvarez I, Uhlmann RA. Anti-Műllerian Hormone (AMH) may stall ovarian cortex function through modulation of hormone receptors other than the AMH receptor. Reproductive Sciences 25(8):1218-1223, 2018. doi: 10.1177/193371911737850. Epub November 15, 2017. PMID: 29141508 Role: Mentor and collaborator
- 12. \*Fletcher NM, \*Abusamaan MS, \*Memai I, \*Saed MG, Al-Hendy A, Diamond MP, Saed GM. Oxidative stress: a key regulator of leiomyoma cell survival. Fertility and Sterility 107(6):1387-1394.e1, 2017. Epub May 5, 2017. PMID: 28483502
- 13. \*Fletcher NM, \*Belotte J, \*Saed MG, \*Memaj I, Diamond MP, Morris RT, Saed GM. Specific point mutations in key redox enzymes are associated with chemoresistance in epithelial ovarian cancer. Free Radical Biology and Medicine 102:122-132, 2017. Epub November 25, 2016. PMID: 27890641
- 14. \*Nusrat O, \*Belotte J, \*Fletcher NM, \*Memaj I, \*Saed MG, Diamond MP, Saed GM. The role of angiogenesis in the persistence of chemoresistance in epithelial ovarian cancer. Reproductive Sciences 23(11):1484-1492, 2016. Epub April 26, 2016. PMID: 27122375
- \*Shaeib F, \*Khan SN, \*Thakur M, Kohan-Ghadr HR, Drewlo S, Saed GM, Pennathur S, Abu-Soud HM. The impact of myeloperoxidase and activated macrophages on metaphase II mouse oocyte quality. PLoS One 11(3):e0151160, 2016. eCollection 2016. PMID: 26982351

Role: Mentor and collaborator

- 16. \*Fletcher NM, Awonuga AO, \*Abusamaan MS, \*Saed MG, Diamond MP, Saed GM. Adhesion phenotype manifests an altered metabolic profile favoring glycolysis. Fertility and Sterility 105(6):1628-1637, 2016. Epub February 23, 2016. PMID: 26920255
- 17. Saed GM, \*Fletcher NM, Diamond MP. The creation of a model for ex vivo development of postoperative adhesions. Reproductive Sciences 23(5):610-622, 2016. September 25, 2015. PMID: 26408397
- 18. \*Fletcher NM, Awonuga AO, \*Neubauer BR, \*Abusamaan MS, \*Saed MG, Diamond MP, Saed GM. Shifting anaerobic to aerobic metabolism stimulates apoptosis through modulation of redox balance: potential intervention in the pathogenesis of postoperative adhesions. Fertility and Sterility 104(4):1022-1029, 2015. Epub July 26, 2015. PMID: 26215756
- 19. \*Belotte J, \*Fletcher NM, \*Saed MG, \*Abusamaan MS, \*Dyson G, Diamond MP, Saed GM. A single nucleotide polymorphism in catalase is strongly associated with ovarian cancer survival. PLoS One 10(8):e0135739, 2015. eCollection 2015. PMID: 26301412 PMCID: PMC4547699

20. \*Belotte J, \*Fletcher NM, \*Alexis M, Morris RT, Munkarah AR, Diamond MP, Saed GM. Sox2 gene amplification significantly impacts overall survival in serous epithelial ovarian Reproductive Sciences 22(1):38-46, 2015. Epub July 18, 2014. PMID: 25038052 PMCID: PMC4275450

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- Role: Mentor, advisor, collaborator, assisted with research
- \*Khan SN, \*Shaeib F, \*Najafi T, \*Kavdia M, Gonik B, Saed GM, Goud PT, Abu-Soud HM. Diffused intra-oocyte hydrogen peroxide activates myeloperoxidase and deteriorates oocyte quality. PLoS One 10(7):e0132388, 2015. eCollection 2015. PMID: 26197395 PMCID: PMC4511228 Role: Mentor, collaborator, assisted with experimental format
- 22. \*Shaeib F, \*Khan SN, \*Ali I, \*Najafi T, \*Maitra D, \*Abdulhamid I, Saed GM, Pennathur S, Abu-Soud HM. Melatonin prevents myeloperoxidase heme destruction and the iron mediated by self-generated hypochlorous acid. PLoS One generation of free 10(3):e0120737, 2015. eCollection 2015. PMID: 25835505 PMCID: PMC4383586 Role: Mentor, collaborator, assisted with writing of article
- \*Maitra D, \*Ali I, \*Abdulridha RM, \*Shaeib F, \*Khan SN, Saed GM, Pennathur S, Abu-23. Kinetic studies on the reaction between dicyanocobinamide and hypochlorous acid. PLoS One 9(11):e110595, 2014. PMID: 25375773 PMCID: PMC4222763
  - Role: Mentor, collaborator, assisted with manuscript writing
- 24. Abu-Soud HM, \*Maitra D, \*Shaeib F, \*Khan SN, \*Byun J, \*Abdulhamid I, \*Yang Z, Saed GM, Diamond MP, Andreana PR, Pennathur S. Disruption of heme-peptide covalent cross-linking in mammalian peroxidases by hypochlorous acid. Journal of Inorganic Biochemistry 140:245-254, 2014. Epub July 8, 2014. PMID: 25193127 PMCID: PMC4449957

Role: Collaborator

- 25. Goud PT, Goud AP, \*Najafi T, Gonik B, Diamond MP, Saed GM, Zhang X, Abu-Soud HM. Direct real-time measurement of intra-oocyte nitric oxide concentration in vivo. PLoS One 9(6):e98720, 2014. PMID: 24887331 PMCID: PMC4041775 Role: Collaborator and assisted with manuscript writing
- 26. \*Fletcher NM, \*Saed MG, \*Abuanzeh S, Abu-Soud HM, Al-Hendy A, Diamond MP, Saed GM. Nicotinamide adenine dinucleotide phosphate oxidase is differentially regulated in normal myometrium versus leiomyoma. Reproductive Sciences 21(9):1145-1152, 2014. Epub February 11, 2014. PMID: 24520084
- \*Fletcher NM, \*Abuanzeh S, \*Saed MG, Diamond MP, Abu-Soud HM, Saed GM. 27. Nicotinamide adenine dinucleotide phosphate oxidase expression is differently regulated to favor a pro-oxidant state that contributes to postoperative adhesion development. Reproductive Sciences 21(8):1050-1059, 2014. Epub February 10, 2014. PMID: 24516041
- Detti L, \*Uhlmann RA, \*Zhang J, Diamond MP, Saed GM, \*Fletcher NM, Lu M, Williams LJ. Goserelin fosters bone elongation, but does not prevent ovarian damage in cyclophosphamide-treated prepubertal mice. Fertility and Sterility 101(4):1157-1164.e.1, 2014. Epub January 23, 2014. PMID: 24462062

Role: Collaborator and mentor

\*Fletcher NM, Awonuga AO, \*Saed MG, Abu-Soud HM, Diamond MP, Saed GM. Lycopene, a powerful antioxidant, significantly reduces the development of the adhesion phenotype. Systems Biology in Reproductive Medicine 60(1):14-20. 2014. Epub November 12, 2013. PMID: 24219141

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- \*†Belotte J, \*†Fletcher NM, Awonuga AO, \*Alexis M, Abu-Soud HM, Saed MG, Diamond MP, Saed GM. The role of oxidative stress in the development of Cisplatin resistance in cancer. †Denotes co-authorship. Reproductive Sciences ovarian 21(4):503-508, 2014. Epub September 27, 2013. PMID: 24077440 PMCID: PMC3960837
- 31. Detti L,\* Uhlmann RA, Lu M, Diamond MP, Saed GM, \*Fletcher NM, Zhang J, Williams LJ. Serum markers of ovarian reserve and ovarian histology in adult mice treated with cyclophosphamide in pre-pubertal age. Journal of Assisted Reproduction and Genetics 30(11):1421-1429, 2013. Epub September 24, 2013. PMID: 24057193 PMCID: PMC3879939. Role: Collaborator and mentor, assisted with experimental design
- Nair S, Saed GM, Atta HM, Rajaratnam V, Diamond MP, Curiel DT, Al-Hendy A. 32. Towards gene therapy of postoperative adhesions: fiber and transcriptional modifications enhance adenovirus targeting towards human adhesion cells. Gynecologic and Obstetric Investigation 76(2):119- 124, 2013. Epub August 6, 2013. PMID: 23920223

Role: Collaborator

- \*Fletcher NM, \*Saed MG, Abu-Soud HM, Al-Hendy A, Diamond MP, Saed GM. Uterine fibroids are characterized by an impaired antioxidant cellular system: potential role of hypoxia in the pathophysiology of uterine fibroids. Journal of Assisted Reproductive Genetics 30(7):969-974, 2013. Epub June 30, 2013. PMID: 23812845
- Detti L, Uhlmann RA, \*Fletcher NM, Diamond MP, Saed GM. Endometrial signaling pathways during ovarian stimulation for assisted reproduction technology. Fertility and Sterility 100(3): 889-894, 2013. Epub June 24, 2013. PMID: 3806847 PMCID: PMC3880797
- \*Banerjee J, \*Shaeib F, \*Maitra D, **Saed GM**, Dai J, Diamond MP, Abu-Soud HM. Peroxynitrite affects the cumulus cell defense of metaphase II mouse oocytes leading to disruption of the spindle structure in vitro. Fertility and Sterility 100(2):578-2013. Epub May 28, 2013. PMID: 23721714 Role: Mentor and collaborator
- 36. \*Maitra D, \*Shaeib F, \*Abdulhamid I, \*Abdulridha RM, Saed GM, Diamond MP, Pennathur S, Abu-Soud HM. Myeloperoxidase acts as a source of free iron during steady-state catalysis by a feedback inhibitory pathway. Free Radical and Biological Medicine 63:90-98, 2013. Epub April 25, 2013. PMID: 23624305, PMCID: PMC3863623

Role: Mentor and collaborator

37. Abu-Soud HM, \*Maitra D, \*Byun J, \*Souza CE, \*Banerjee J, Saed GM, Diamond MP, Andreana PR, Pennathur S. The reaction of HOCI and cyanocobalamin: corrin destruction and the liberation of cyanogen chloride. Free Radical and Biological Medicine 52(3):616-625, 2012. Epub November 10, 2011. PMID: 22138102 PMCID: PMC3786219

Role: Mentor and collaborator

- 38. \*Ambler DR, \*Fletcher NM, Diamond MP, **Saed GM**. Effects of hypoxia on the expression of inflammatory markers IL-6 and TNF-α in human normal peritoneal and adhesion fibroblasts. Systems Biology and Reproductive Medicine 58(6):324-329, 2012. Epub October 8, 2012. PMID: 2304632
- 39. \*Maitra D, \*Abdeulhamid I, Diamond MP, **Saed GM**, Abu-Soud HM. Melatonin attenuates hypochlorous acid-mediated heme destruction, free iron release, and protein aggregation in hemoglobin. Journal of Pineal Research 53(2):198-205, 2012. Epub March 30, 2012. PMID: 22462755

Role: Mentor and collaborator

- 40. \*Ambler DR, \*Golden AM, \*Gell JS, Saed GM, Carey DJ, Diamond MP. Microarray expression profiling in adhesion and normal peritoneal tissues. Fertility and Sterility 97(5):1158-1164.e1-4, 2012. Epub February 22, 2012. PMID: 22365381 Role: Mentor, collaborator, assisted with writing of article
- 41. \*Shavell VI, \*Fletcher NM, \*Jiang ZL, Saed GM, Diamond MP. Uncoupling oxidative phosphorylation with 2,4-dinitrophenol promotes development of the adhesion phenotype. Fertility and Sterility 97(3):729-733, 2012. Epub December 24, 2011. PMID: 22200174

Role: Mentor and collaborator; assisted with experimental design

42. Abu-Soud HM, \*Maitra D, \*Byun J, \*Souza CE, \*Banerjee J, Saed GM, Diamond MP, Andreana PR, Pennathur S. The reaction of HOCl and cyanocobalamin: corrin destruction and the liberation of cyanogens chloride. Free Radical Biology and Medicine 52(3):616-625, 2012. Epub November 10, 2011. PMID: 22138102 PMCID: PMC3786219

Role: Mentor and collaborator

43. \*Souza CE, \*Maitra D, Saed GM, Diamond MP, Moura AA, Pennathur S, Abu-Soud HM. Hypochlorous acid-induced heme degradation from lactoperoxidase as a novel mechanism of free iron release and tissue injury in inflammatory diseases. PLoS One 6(11):e27641, 2011. Epub November 22, 2011. PMID: 22132121 PMCID: PMC3222650

Role: Mentor and collaborator

- 44. \*White JC, \*Jiang ZL, Diamond MP, Saed GM. Macrophages induce the adhesion phenotype in normal peritoneal fibroblasts. Fertility and Sterility 96(3):758-763.e3, 2011. Epub July 27, 2011. PMID: 21794857
- \*Maitra D, \*Byun J, \*Andreana PR, Abdulhamid I, Diamond MP, Saed GM, Pennathur S, 45. Abu-Soud HM. Reaction of hemoglobin with HOCI: mechanism of heme destruction and free iron release. Free Radical Biology and Medicine 51(2):374-386, 2011. Epub April 3, 2011. PMID: 21549834 PMCID: PMC3863628 Role: Mentor and collaborator
- \*Maitra D, Byun J, \*Andreana PR, Abdulhamid I, Saed GM, Diamond MP, Pennathur S, Abu-Soud HM. Mechanism of hypochlorous acid-mediated heme destruction and free iron release. Free Radical Biology and Medicine 51(2):364-373, 2011. Epub April 3, 2011. PMID: 21466849 PMCID: PMC3378337 Role: Mentor and collaborator
- Saed GM, \*Fletcher NM, \*\*Jiang ZL, Abu-Soud HM, Diamond MP. Dichloroacetate induces apoptosis of epithelial ovarian cancer cells through a mechanism involving modulation of oxidative stress. Reproductive Sciences 18(12):1253-1261, 2011. Epub June 23, 2011. PMID: 21701041
- \*Meng Q, \*Sun W, \*Jiang J, \*Fletcher NM, Diamond MP, Saed GM. Identification of common mechanisms between endometriosis and ovarian cancer. Journal of Assisted Reproduction and Genetics 28(10):917-923, 2011. Epub May 26, 2011. PMID: 21614520 PMCID: PMC3220443

49. \*Jiang Z, \*Fletcher NM, \*Ali-Fehmi R, Diamond MP, Abu-Soud HM, Munkarah AR, **Saed GM**. Modulation of redox signaling promotes apoptosis in epithelial ovarian cancer cells. Gynecologic Oncology 122(2):418-423, 2011. Epub May 26, 2011. PMID: 21620448 PMCID: PMC4237166

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- 50. Detti L, **Saed GM**, \*Fletcher NM, Kruger ML, Brossoit M, Diamond MP. Endometrial morphology and modulation of hormone receptors during ovarian stimulation for assisted reproductive technology cycles. Fertility and Sterility 95(3):1037-1041, 2011. Epub January 12, 2011. PMID: 21227412 PMCID: PMC3769103

  Role: Collaborator and mentor; assisted with experimental design
- 51. \*Fletcher NM, \*Jiang Z, \*Ali-Fehmi R, \*Levin NK, \*Belotte J, Tainsky MA, Diamond MP, Abu-Soud HM, **Saed GM**. Myeloperoxidase and free iron levels: potential biomarkers for early detection and prognosis of ovarian cancer. Cancer Biomarkers 10(6):267-275, 2011-2012. PMID: 22820082
- 52. \*Ali-Fehmi R, \*Semaan A, \*Sethi S, \*Arabi H, \*Bandyopadhyay S, \*Hussein R, Diamond MP, **Saed GM**, Morris RT, Munkarah AR. Molecular typing of epithelial ovarian carcinomas using inflammatory markers. Cancer 117(2):301-309, 2011. Epub September 3, 2010. PMID: 20818651

  Role: Collaborator, mentor and assisted with research protocol
- 53. Bryant CS, Munkarah AR, Kumar S, Batchu RB, Shah JP, Berman J, Morris RT, \*Jiang ZL, **Saed GM**. Reduction of hypoxia-induced angiogenesis in ovarian cancer cells by inhibition of HIF-1 alpha gene expression. Archives of Gynecology and Obstetrics 282(6):677-683, 2010. Epub February 7, 2010. PMID: 20140681
- 54. Pennathur S, \*Maitra D, \*Byun J, \*Sliskovic I, \*Abdulhamid I, **Saed GM**, Diamond MP, Abu-Soud HM. Potent antioxidative activity of lycopene: a potential role in scavenging hypochlorous acid. Free Radical Biology and Medicine 49(2):205-213, 2010. Epub April 11, 2010. PMID: 20388538 PMCID: PMC3416054 Role: Collaborator and mentor
- 55. **Saed GM**, \*Jiang ZL, \*Fletcher NM, \*Al Arab A, Diamond MP, Abu-Soud HM. Exposure to polychlorinated biphenyls enhances lipid peroxidation in human normal peritoneal and adhesion fibroblasts: a potential role for myeloperoxidase. Free Radical Biology and Medicine 48(6):845-850, 2010. Epub January 11, 2010. PMID: 20067832 PMCID: PMC2834263
- 56. **Saed GM**, \*Ali-Fehmi R, \*Jiang ZL, \*Fletcher NM, Diamond MP, Abu-Soud HM, Munkarah AR. Myeloperoxidase serves as a redox switch that regulates apoptosis in epithelial ovarian cancer. Gynecologic Oncology 116(2):276-281, 2010. Epub December 3, 2009. PMID: 19962178 PMCID: PMC2834266
- 57. \*Jiang ZL, \*Fletcher NM, Diamond MP, Abu-Soud HM, **Saed GM**. S-nitrosylation of caspase-3 in the mechanism by which adhesion fibroblasts manifest lower apoptosis. Wound Repair and Regeneration 17(2):224-229, 2009. PMID: 19320891 PMCID: PMC4529118

58. \*Jiang ZL, \*Fletcher NM, Diamond MP, Abu-Soud HM, Saed GM. Hypoxia regulates iNOS expression in human normal peritoneal and adhesion fibroblasts through nuclear factor kappa B activation mechanism. Fertility and Sterility 91(2):616-621, 2009.

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59. Saed GM, \*Jiang Z, Diamond MP, Abu-Soud HM. The role of myeloperoxidase in the pathogenesis of postoperative adhesions. Wound Repair and Regeneration 17(4):531-539, 2009. PMID: 19614918

Epub February 20, 2008. PMID: 18281043 PMCID: PMC2812021

- 60. Saed GM, \*Jiang Z, \*Fletcher NM, Diamond MP. Modulation of the BCL-2/BAX ratio by interferon-gamma and hypoxia in human peritoneal and adhesion fibroblasts. Fertility and Sterility 90(5):1925-1930, 2008. Epub October 1, 2007. PMID: 17905238
- 61. \*Jiang ZL, Zhu X, Diamond MP, Abu-Soud HM, Saed GM. Nitric oxide synthase isoforms expression in fibroblasts isolated from human normal peritoneum and adhesion tissues. Fertility and Sterility 90(3):769-764, 2008. Epub April 26, 2008. PMID: 18440510 PMCID: PMC2574925
- 62. \*Fletcher NM, \*Jiang ZL, Diamond MP, Abu-Soud HM, Saed GM. Hypoxia-generated superoxide induces the development of the adhesion phenotype. Free Radical Biology and Medicine 45(4):530-536, 2008. Epub May 15, 2008. PMID: 18538674 PMCID: PMC2574925
- 63. \*Detti L, Saed GM, Jiang ZL, Kruger ML, Diamond MP. The effect of estradiol on the expression of estrogen, progesterone, androgen, and prolactin receptors in human peritoneal fibroblasts. Journal of Assisted Reproduction and Genetics 25(6):245-250, 2008. Epub June 13, 2008. PMID: 18551363 PMCID: PMC2582083 Role: Mentor and collaborator, assisted with research protocol and writing of article
- 64. Diamond MP, Wirth JJ, Saed GM. PCBs enhance collagen I expression from human peritoneal fibroblasts. Fertility and Sterility 90(4 Suppl):1372-1375, 2008. Epub September 20, 2007. PMID: 17888429 PMCID: PMC2603182
- 65. Saed GM, Al-Hendy A, Salama SA, Diamond MP. Adenovirus-mediated expression of cyclooxygenase-2 antisense reverses abnormal genetic profile of human adhesion fibroblasts. Fertility and Sterility 89(5 Suppl):1455-1460, 2008. Epub July 10, 2007. PMID: 17624339
- 66. **Saed GM**. Immunohistochemical staining of cyclooxygenases with monoclonal antibodies. Methods in Molecular Biology 477:219-228, 2008. PMID: 19082950
- 67. Pansare V, Munkarah AR, \*Schimp V, Haitham Arabi M, Saed GM, Morris RT, Ali-Fehmi R. Increased expression of hypoxia-inducible factor 1-alpha in type I and type II endometrial carcinomas. Modern Pathology 20(1):35-43, 2007. Epub November 10, 2006. PMID: 17099695 Role: Collaborator
- 68. \*Alpay Z, \*Ozgönenel M, \*Savasan S, \*Buck S, **Saed GM**, Diamond MP. Altered in vitro immune response to hypoxia-treated normal peritoneal fibroblasts. Fertility and Sterility 87(2):426-429, 2007. Epub November 15, 2006. PMID: 17113091

Role: Mentor, collaborator

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## **Book Authorships, Editorships, and Chapters**

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## **Non Peer-Reviewed Publications**

### Other (On-Line Publications) \* Indicates student, trainee, or postdoctoral

- \*Nusrat O, \*Belotte J, \*Fletcher NM, \*Memaj I, \*Saed MG, Diamond MP, Saed GM. The 1. role of angiogenesis in the persistence of chemoresistance in epithelial ovarian cancer. www.OncToday.com, Beyond the Abstract, June 21, 2016.
- 2. \*Belotte J, \*Fletcher NM, \*Alexis M, Morris RT, Munkarah AR, Diamond MP, Saed GM. Sox2 gene amplification significantly impacts overall survival in serous epithelial ovarian cancer. Global Medical Discovery Series (Key Scientific Article Contributing to Excellence in Biomedical Research), summer issue 2015.
- \*Fletcher NM, \*Saed MG, Abu-Soud HM, Al-Hendy A, Diamond MP, Saed GM. Uterine 3. fibroids are characterized by an impaired antioxidant cellular system: potential role of hypoxia in the pathophysiology of uterine fibroids. Featured article. MDLinx.com/ obstetrics-gynecology/ news-article, November 2013.

#### **PRESENTATIONS**

# **Podium Presentations (Referred)**

- 1. Cyclophosphamide and Its Metabolite Impact on Fertilization through Mitochondrial *Dysfunction*. 65<sup>th</sup> Annual Meeting of the Pacific Coast Reproductive Society, Indian Wells, CA, March 2017.
- Novel Target for Ovarian Cancer Immunotherapy. 48th Annual Meeting of the Society of 2. Gynecologic Oncology's Women's Cancer, National Harbor, MD, March 2017.
- 3. Targeting Integrin αV/β1 Receptor Manifests Intriguing Anti-Tumor Effects in Sensitive and Chemoresistant Ovarian Cancer Cells: Potential Therapeutic Target. Annual Scientific Meeting of the Society for Reproductive Investigation, Orlando, FL. March 2017.
- 4. Abciximab Manifests Striking Anti-Tumor Effects in Sensitive and Chemoresistant Ovarian Cancer Cells. Ghassan M. Saed PhD, Nicole M. Fletcher PhD, Ira Memaj BS Wayne State University School, Department of Obstetrics and Gynecology, Detroit, MI. Reproductive Sciences Retreat and MARTS 2017

5. Human Adhesion Fibroblasts are Characterized by Reduction in the level of Pluripotency Markers as Compared to Normal Peritoneal Fibroblasts. 72<sup>nd</sup> Annual Meeting of the American Society for Reproductive Medicine, Salt Lake City, UT, October 2016.

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- 6. Anti-Mullerian Hormone (AMH) for Prevention of Tissue Activation after Vitrified/Thawed Ovarian Cortex Xenotransplantation. 72nd Annual Meeting of the American Society for Reproductive Medicine, Salt Lake City, UT, October 2016.
- 7. Dichloroacetate Induces Apoptosis of Uterine Leiomyoma Cells Through A Mechanism Involving Modulation of Oxidative Stress. 63rd Annual Meeting of the Society for Reproductive Investigation, Montreal, Quebec, Canada, March 2016.
- 8. Chemoresistance in Epithelial Ovarian Cancer Cells is Controlled by Mechanisms Emanating from Chemotherapy-Induced Genotype Switch in Glutathione Peroxidase, Through the Up-Regulation of Cytidine Deminase. 62<sup>nd</sup> Annual Meeting of the Society for Reproductive Investigation, San Francisco, CA, March 2015.
- 9. Elevated Serum Anti-Müllerian Hormone (AMH) Stalls Ovarian Follicle Development by Downregulating FSH- and LH-Receptors and Inhibin-B Production. Proceedings of the 71st Annual Meeting of the American Society for Reproductive Medicine, Baltimore, MD, October 2015.
- 10. Hypochlorous Acid Reversibly Inhibits Caspase-3: A Potential Regulator of Apoptosis. Joint Meeting of the 22<sup>nd</sup> Society for Redox Biology and Medicine (SFRBM) and 17<sup>th</sup> Society for Free Radical Research International (SFRRI), Boston, MA, November 2015.
- 11. The In-Vivo Effects of Superoxide Dismutase on the Incidence and Severity of Post-Operative Adhesion Development. 70th Annual Meeting of the American Society for Reproductive Medicine, Honolulu, HI, October 2014.
- 12. Superoxide Dismutase Significantly Delayed the Development of Cisplatin Resistance in Epithelial Ovarian Cancer Cells. American Association for Cancer Research's Precision Medicine Series: Drug Sensitivity and Resistance. Improving Cancer Therapy Special Conference, Orlando, FL, June 2014.
- Chemoresistant Ovarian Cancer Cells Manifest Lower Vascular Endothelial Growth 13. Factor and Hypoxia Induced Factor-1α: A Potential Survival Mechanism. American Association for Cancer Research's Precision Medicine Series: Drug Sensitivity and Resistance. Improving Cancer Therapy Special Conference, Orlando, FL, June 2014.
- 14. Dicholoroacetate Increases Sensitivity to Chemotherapy by Modulation of Antioxidants in Epithelial Ovarian Cancer. 61st Annual Meeting of the Society for Gynecologic Investigation, Florence, Italy, March 2014.
- 15. Catalase and NADPH Oxidase Single Nucleotide Polymorphisms Are Associated with Increased Risk and Serve As Potential Targets for Breast and Ovarian Cancers. 104th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2013.

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- 16. The Role of Oxidative Stress in the Development of Cisplatin Resistance in Epithelial Ovarian Cancer. Poster session B. Advances in Ovarian Cancer Research: From Concept to Clinic. American Association for Cancer Research, Miami, FL, September 2013.
- Catalase and NADPH Oxidase Single Nucleotide Polymorphisms Are Associated with Increased Risk and Serve As Potential Targets for Breast and Ovarian Cancers. 104th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2013.
- Endometrial Insulin Pathway during Ovarian Stimulation for Assisted Reproductive Technology (ART). 68th Annual Meeting of the American Society for Reproductive Medicine, San Diego, CA, October 2012.
- 19. NADPH Oxidase p22-Phox Gene Polymorphism in Women is Associated with the Development of Postoperative Adhesions. 59th Annual Meeting of the Society for Gynecologic Investigation, San Diego, CA, March 2012.
- 20. Metabolism and Oxidative Stress: Integral Role in Regulation of the Adhesion Phenotype. 58th Annual Meeting of the Society for Gynecologic Investigation, Miami Beach, FL, March 2011.
- 21. Mass Spectrometric Identification of HOCI-Mediated Heme Degradation Products of Hemoglobin. 59th ASMS Conference on Mass Spectrometry, Denver, CO, 2011.
- 22. Inhibition of NADPH Oxidative Reductase Promotes Apoptosis in Epithelial Ovarian Cancer Cells. 39th Annual Meeting of the Global Congress of Minimally Invasive Gynecology AAGL, Las Vegas, NV, November 2010.
- 23. Reaction of Hemoglobin and Red Blood Cells with Hypochlorous Acid and Mechanism of Heme Destruction and Free Iron Release. 17th Annual Meeting of the Society for Free Radical Biology and Medicine, Orlando, FL, November 2010.
- 24. Liquid Chromatography Atmospheric Pressure Ionization Tandem: Mass Spectrometry Identifies Novel Hypochlorous Acid Reaction Products of Lycopene. 58th Annual Meeting of the American Society of Mass Spectrometry, Salt Lake City, UT, May 2010.
- 25. Role of Polychlorinated Biphenyls Enhancement of Lipid Peroxidation in Human Normal Peritoneal and Adhesion Fibroblasts. 38th Annual Meeting of Global Congress of Minimally Invasive Gynecology AAGL, Orlando, FL, November 2009.
- 26. Hydrogen Peroxide Bioavailability Determines the Sensitivity of Human Normal Peritoneal and Adhesion Fibroblasts to Hypoxia-Induced Lipid Peroxidation. 38th Annual Meeting of Global Congress of Minimally Invasive Gynecology AAGL, Orlando, FL, November 2009.
- 27. S-Nitrosylation of Caspase-3 Is the Mechanism by Which Adhesion Fibroblasts Manifest Lower Apoptosis. 36th Annual Meeting of the American Association of

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- Gynecologic Laparoscopists, Global Congress of Minimally Invasive Gynecology, Washington, DC, November 2007.
- 28. Generation of Superoxide by Inducible Nitric Oxide Synthase in L-Arginine Deficient Fibroblasts Established From Human Adhesion Tissues. 36th AAGL Annual Meeting. Global Congress of Minimally Invasive Gynecology, Washington, DC, November 2007.

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- 29. Hypoxia Stimulation of Expression of Type I Collagen and Fibronectin in Human Peritoneal and Adhesion Fibroblasts: Blockage by Interferon Gamma. 36th AAGL Annual Meeting, Global Congress of Minimally Invasive Gynecology, Washington, DC, November 2007.
- Superoxide Induces the Adhesion Phenotype: Role of Hypoxia in the Pathogenesis of the Adhesion Development. Global Congress of Minimally Invasive Gynecology, 36th Annual Meeting of the American Association of Gynecologic Laparoscopists, Washington, DC, November 2007.
- 31. Nitric Oxide Synthase Isoforms are Differentially Expressed in Fibroblasts Isolated from Human Normal Peritoneum and Adhesion Tissues. 63rd Annual Meeting of the American Society for Reproductive Medicine, Washington, DC, October 2007.
- Regulation of the Expression of INOS, COX-2, and VEGF in Postoperative Adhesions. 62<sup>nd</sup> Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.
- 33. Omega-3 Fatty Acid Prevents and Mitigates the Adhesion Phenotype in Normal Human Peritoneal and Adhesion Fibroblasts. 62<sup>nd</sup> Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.
- 34. IL6 Expression in Human Normal Peritoneal and Adhesion Fibroblasts: Regulation by Hypoxia. 62<sup>nd</sup> Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.
- The Cross-Talk between Myeloperoxidase and Inducible Nitric Oxide Synthase in Post-35. operative Adhesions. 62<sup>nd</sup> Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.
- 36. TNF-Alpha Expression in Human Normal Peritoneal and Adhesion Fibroblasts: Regulation by Hypoxia. 62<sup>nd</sup> Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.
- 37. L-Arginine Deficiency in Fibroblasts Established from Human Adhesion Tissues Leads to the Generation of Superoxide by Inducible Nitric Oxide Synthase. 53rd Annual Meeting of the Society for Gynecologic Investigation, Toronto, Ontario, Canada, March 2006.
- Regulation of Inducible Nitric Oxide Synthase in Post-Operative Adhesions. 34th Annual 38. Meeting of the American Association of Gynecologic Laparoscopists, Chicago, IL, November 2005.

Cyclooxygenase-2 Inhibitors Enhance Apoptosis of Adhesion Fibroblasts. 34<sup>th</sup> Annual Meeting of the American Association of Gynecologic Laparoscopists, Chicago, IL, 39. November 2005.

40. The Effects of Estradiol on the Expression of Estrogen, Progesterone, Androgen, and Prolactin Receptors in Human Peritoneal Fibroblasts. 61st Annual Meeting of the American Society for Reproductive Medicine and the 1st Annual Meeting of the Canadian Fertility and Andrology Society, Palais des Congres, Montreal, Quebec, Canada, October 2005.

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- 41. Possible Role of Natural Immune Response against Fibroblasts in the Development of Post-Operative Adhesions. 61st Annual Meeting of the American Society for Reproductive Medicine and the 51st Annual Meeting of the Canadian Fertility and Andrology Society, Palais des Congres, Montreal, Quebec, Canada, October 2005.
- Knockout of Inducible Nitric Oxide Expression Significantly Reduces the Expression of 42. Type I Collagen and Transforming Growth Factor-β1 in Human Peritoneal and Adhesion Fibroblasts. 61st Annual Meeting of the American Society for Reproductive Medicine and the 51st Annual Meeting of the Canadian Fertility and Andrology Society, Palais des Congres, Montreal, Quebec, Canada, October 2005. Prize Paper Candidate
- 43. Regulation of Inducible Nitric Oxide Synthase in Post-Operative Adhesions. 52<sup>nd</sup> Annual Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2005.
- 44. Differential Expression of Myeloperoxidase (MPO) in Fibroblasts Isolated from Normal Peritoneal and Adhesion Tissues. 4th International Peroxidase Meeting Joint with the 10th Myeloperoxidase Meeting, Shimokyo-Ku, Kyoto City, Japan, October 2004.
- 45. Fibroblasts Isolated from Normal Peritoneal and Adhesion Tissues Differentially Express Myeloperoxidase (MPO). 60th Annual Meeting of the American Society for Reproductive Medicine, Philadelphia, PA, October 2004.
- 46. Hypoxia Up-Regulates Cyclooxygenase-2 and Prostaglandin E2 Levels in Human Peritoneal Fibroblasts. 60th Annual Meeting of the American Society for Reproductive Medicine, Philadelphia, PA, October 2004.
- 47. Dichloroacetate Inhibition of Angiogenesis Caused by Hypoxia Treatment of Normal Peritoneal and Adhesion Fibroblasts in Human Umbilical Vein Endothelial Cells. 60th Annual Meeting of the American Society for Reproductive Medicine, Philadelphia, PA, October 2004.
- 48. Dichloroacetate Significantly Increase the Expression of the Transcription Nuclear Factor Kappa-β in Fibroblasts of Human Adhesion Tissues. 51st Annual Scientific Meeting of the Society for Gynecologic Investigation, Houston, TX, March 2004.
- 49. Stimulation of Expression of Vascular Endothelial Growth Factor by Hypoxia from Fibroblasts Isolated from Normal Peritoneum and Adhesion Tissues. 32<sup>nd</sup> Annual Meeting of The American Association of Gynecologic Laparoscopists, Las Vegas, NV, November 2003.
- 50. Inhibition of Nitric Oxide Production by N-Nitro-L-Arginine Methyl Ester Increased the Expression of Type I Collagen in Human Peritoneal Fibroblasts. 59th Annual Meeting of American Society for Reproductive Medicine, San Antonio, TX, October 2003.

51. Apoptosis of Human Peritoneal and Adhesion Fibroblasts After Hypoxia: Role of Inducible Nitric Oxide Synthase. 59th Annual Meeting of American Society for Reproductive Medicine, San Antonio, TX, October 2003.

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- 52. Inhibition of Cyclooxygenase-2 in Fibroblasts Isolated from Normal Peritoneum and Adhesion Tissues Decreases the Expression of Hypoxia Inducible Factor-1 Alpha. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.
- 53. Tissue Plasminogen Activator/Plasminogen Activator Inhibitor-1 (tPA/PAI-1) Modulation by Tisseel. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.
- 54. Hypoxia Increases the Expression of Vascular Endothelial Growth Factor in Fibroblasts Isolated From Human Normal Peritoneum and Adhesion Tissues. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.
- 55. Dichloroacetate Significantly Reduces the Expression of Vascular Endothelial Growth Factor in Fibroblasts of Human Adhesion Tissues. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.
- 56. Transforming Growth Factor-Beta 1 (TGF-\(\beta\)1) and Extracellular Matrix Production by Human Peritoneal Mesothelial Cells: Effect of Tisseel® Fibrin Sealant). 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.
- 57. Cyclooxygenase-2 Inhibition Decreases the Expression of Vascular Endothelial Growth Factor from Fibroblasts Isolated from Normal Peritoneum and Adhesion Tissues. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.
- 58. Elevation of Type I Collagen mRNA in Peritoneal Adhesions. 31st Annual Meeting of the American Association of Gynecologic Laparoscopists, Miami, FL, November 2002.
- 59. Cyclooxygenase-2 Expression in Human Fibroblasts Isolated from Adhesions But Not from Normal Peritoneal Tissues. 31st Annual Meeting of the American Association of Gynecologic Laparoscopists, Miami, FL, November 2002.
- 60. Existence of p53 Expression in Human Fibroblasts Isolated from Adhesions, But Not from Normal Peritoneal Tissues. 31st Annual Meeting of the American Association of Gynecologic Laparoscopists, Miami, FL, November 2002.
- 61. Matrix Metalloproteinase (MMP-1, MMP-2), and Tissue Inhibitor for Metalloproteinase (TIMP-1) Expression by Human Peritoneal Mesothelial Cells: Effect of Fibrin Sealant. 58th Annual Meeting of the American Society for Reproductive Medicine, Seattle, WA, October 2002.

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- 62. Dichloroacetate (DCA) Significantly Increases the Expression of Inducible Nitric Oxide Synthase (INOS) in Human Fibroblasts of Adhesion Tissues, But Not In Normal Peritoneum. 58th Annual Meeting of the Society for Reproductive Medicine, Seattle, WA, October 2002.
- 63. Seprafilm (Modified Hyaluronic Acid Carboxymethylcellulose) Acts as a Mechanical Barrier, 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.
- 64. Inhibition of Cyclooxygenase-2 in Human Adhesion Fibroblasts Reduces the Expression of MMP-1 and TIMP-1. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.
- 65. Inhibition of Cyclooxygenase-2 in Human Adhesion Fibroblasts Reduces the Expression of Transforming Growth Factor Beta-1. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.
- 66. Adhesion Phenotype: Cyclooxygenase-2 is Expressed in Fibroblasts Isolated From Adhesions, But Not From Normal Peritoneal Tissues. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.
- 67. Reduction of the Expression of Type I and III Collagens in Human Adhesion Fibroblasts, But Not in Normal Peritoneal Fibroblasts by the Inhibition of Cyclooxygenase-2. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.
- 68. Dichloroacetate Significantly Reduces the Expression of Cyclooxygenase-2 in Human Fibroblasts of Adhesion Tissues. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.
- 69. Adhesion Phenotype: p53 is Expressed in Fibroblasts Isolated From Adhesions But Not From Normal Peritoneal Tissues. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.
- 70. Metabolic Regulation of Collagen I in Fibroblasts Isolated from Normal Peritoneum and Adhesions by Dichloroacetic Acid (DCA). 28th Scientific Meeting of Gynecologic Surgeons, Dallas, TX, March 2002.
- 71. An Adhesion Promoting Phenotype: Implications for Postoperative Adhesion Development. 30<sup>th</sup> Annual Meeting American Association of Laparoscopists, Global Congress of Gynecologic Endoscopy, San Francisco, CA, November 2001.
- 72. Differences in the Rate of Apoptosis Following Hypoxia in Human Peritoneal and Adhesion Fibroblasts. 30th Annual Meeting American Association of Gynecologic Laparoscopists. Global Congress of Gynecologic Endoscopy, San Francisco, CA, November 2001.

- 73. Modulation of the BCL-2/BAX Ratio by IFN-GAMMA and Hypoxia in Human Peritoneal and Adhesion Fibroblasts. 57th Annual Meeting of the American Society for Reproductive Medicine, Orlando, FL, October 2001.

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- 74. Significance of the Effect of Hypoxia on the Rate of Apoptosis of Human Peritoneal and Adhesion Fibroblasts for Postoperative Adhesion Development. 57th Annual Meeting of the American Society for Reproductive Medicine, Orlando, FL, October 2001.
- 75. Prostaglandin E2 Stimulates Proliferation and Reduces Apoptosis in Epithelial Ovarian Cancer Cell Lines. 48th Annual Meeting of the Society for Gynecologic Investigation. Toronto, Canada, March 2001.
- 76. Differential Modulation of BCL-2/BAX Ratio by Hypoxia in Peritoneal and Adhesion Fibroblasts Cultured from the Same Patient. 48th Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, March 2001.
- 77. Interferon Gamma Blocks the Stimulating Effect of Hypoxia on the Expression of Type I Collagen and Fibronectin in Human Peritoneal and Adhesion Fibroblasts. 48th Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, March 2001.
- 78. The Effect of Interferon Gamma and Hypoxia on the Expression of TGF-\( \mathcal{B} \) Isoforms in Human Peritoneal and Adhesion Fibroblasts. 48th Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, March 2001.
- 79. The Effect of Normoxia after Hypoxia Treatment of the Expression of Type I Collagen and TGF-β1 in Human Peritoneal Fibroblasts: Implications for Postoperative Adhesion Development. 48th Annual Meeting of the Society for Gynecologic Investigation. Toronto, Canada, March 2001.
- Modulation of the BCL-2/BAX Ratio by IFN-γ and Hypoxia in Human Peritoneal and 80. Adhesion Fibroblasts. 32nd Annual Meeting of the Society of Gynecologic Oncologists, Nashville, TN, February 2001.
- Prostaglandin in Induced COX-2 Expression and Reduced Apopotosis in Epithelial 81. Ovarian Cancer Cells. 32nd Annual Meeting of the Society of Gynecologic Oncologists, Nashville, TN, February 2001.
- 82. The Effect of Hypoxia on the Expression of HIF-1β, BAX, and BCL-2 in the Epithelial Ovarian Cancer Cell Line MADH2774. 32nd Annual Meeting of the Society of Gynecologic Oncologists, Nashville, TN, February 2001.
- 83. Induction of Cyclooxygenase-2 by Prostaglandin E₂ in Human Ovarian Cancer Cell Lines. 53rd Congress of the DGGG, German Society of Gynecology and Obstetrics eV, Munich, Germany, June 2000.
- Type I Collagen Production by Human Peritoneal Fibroblasts in Response to Hypoxia 84. and/or Transforming Growth Factor-Beta 1 (TGF-β1) Treatments. 47th Annual Meeting of the Society for Gynecologic Investigation, SGI 2000-A Millennial Milestone in Reproductive Sciences: Celebrating the Promise, Chicago, IL, March 2000.

85. The Effect of Hypoxia on TGF-\$1 on the Expression of Cellular Fibronectin in Human Peritoneal Fibroblast Cells in Culture. 47th Annual Meeting of the Society for Gynecologic Investigation, SGI 2000-A Millennial Milestone in Reproductive Sciences: Celebrating the Promise, Chicago, IL, March 2000.

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- 86. Type I Collagen Expression in Adhesion and Normal Peritoneal Tissues. 47th Annual Meeting of the Society for Gynecologic Investigation, SGI 2000-A Millennial Milestone in Reproductive Sciences: Celebrating the Promise, Chicago, IL, March 2000.
- 87. Vascular Endothelial Growth Factor (VEGF) Levels Are Elevated in Adhesion Tissue in Humans. Annual Meeting of the American Association of Gynecologic Laparoscopists, Las Vegas, NV, November 1999.
- Basics of Cutaneous Wound Repair. 4th International Conference on Postoperative 88. Healing and Adhesions, Fort Lauderdale, FL, October 1999.
- 89. The Role of Extracellular Matrix in the Formation of Postoperative Adhesion. International Conference on Postoperative Healing and Adhesions, Fort Lauderdale, FL, October 1999.
- 90. The Effect of Hypoxia and TGF-β1 on the Expression of Tissue Inhibitors of Metalloproteinases (TIMP-1) in Human Peritoneal Mesothelial Cells. Joint meeting of the Canadian Fertility Society and the American Society for Reproductive Medicine, Toronto, Ontario, Canada, September 1999.
- 91. Collagen Type I and Type III Production by Human Mesothelial Cells in Response to Hypoxia and/or TGF-81 Treatments. Annual Meeting of the Society for Gynecologic Investigation, Atlanta, GA, March 1999.
- 92. The Role of Apoptosis and p53 in the Pathogenesis of Keloids. Journal of Investigative Dermatology 110: 597, 1998.
- 93. Apoptosis Modulation in the Response of CTCL to PUVA. Journal of Investigative Dermatology 110: 698, 1998.
- 94. Apoptosis Dysregulation in Keloid Fibroblasts. Journal of Investigative Dermatology 110:653, 1998.
- 95. Apoptosis Regulation in the Pathogenesis of Cutaneous T-Cell Lymphoma (CTCL). Journal of Investigative Dermatology 108:610, 1997.
- 96. The Effect of PUVA Treatment on HUT78 Cell Differential Gene Expression. Journal of Investigative Dermatology 106:906, 1996.
- 97. Detection of Differentially Displayed cDNA Fragments in Normal vs Sezary Syndrome Leukocytes. Journal of Investigative Dermatology 104: 673, 1995.
- 98. Quantitative PCR Analysis of Th-1 Cytokines in HUT78 Cells after Exposure to PUVA In Vitro. Journal of Investigative Dermatology 102: 585, 1994.

99. Augmentation of Th-1 Cytokines in the Peripheral Blood of Sezary Syndrome Patients after Treatment with ECCP. Journal of Investigative Dermatology 102:586, 1994.

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- 100. Augmentation of Th-1 Cytokines in the Peripheral Blood of SZ Patients Upon Treatment with Extracorporeal Photopheresis. Clinical Research 41:664, 1993.
- 101. Detection of T-Cell Clonality in Mycosis Fungoides by PCR-Metaphore Agarose Analysis of T-Cell Receptor-y. Clinical Research 41:459, 1993.
- 102. Mycosis Fungoides and Psoriasis Exhibit a Th1 Type Cell Mediated Response While Sezary Syndrome Expresses A Th2 Type Response. Clinical Research 40:730, 1992.
- 103. T-Cell Receptor Gene Conservation and Rearranged Clones in Canine Mycosis Fungoides. Clinical Research 40:505, 1992.

# **Poster Presentations (Referred)**

- Fletcher NM, Memaj I, Diamond MP, Morris RT, Saed GM. Heat Shock Protein 60 1. (HSP60) Serves as a Potential Target for the Sensitization of Chemoresistant Ovarian Cancer Cells. 49th Society of Gynecologic Oncology Annual Meeting on Women's Cancer, New Orleans, LA, March 24-27, 2018. Gynecologic Oncology Supplement, 2018.
- 2. Fletcher NM, Memaj I, Diamond MP, Morris RT, Saed GM. Targeting Myeloperoxidase Enhances Apoptosis in Chemoresistant Epithelial Ovarian Cancer Cells by Reversing S-Nitrosylation of Caspase-3. 49th Society of Gynecologic Oncology Annual Meeting on Women's Cancer, New Orleans, LA, March 2018. Gynecologic Oncology Supplement, 2018.
- 3. Fletcher NM, Aownuga AO, Memaj M, Diamond MP, Al-Hendy AA, Saed GM. A Novel Role for the Interaction of Myeloperoxidase and CD11b in Leiomyoma Cells. 65<sup>th</sup> Annual Scientific Meeting for the Society for Reproductive Investigation, San Diego, CA, March 2018. Session Gynecology Proceedings: S-116, 2018.
- 4. Fletcher NM, Memaj I, Saed GM. Talcum Powder Enhances Oxidative Stress in Ovarian 65<sup>th</sup> Annual Scientific Meeting for the Society for Reproductive Investigation, San Diego, CA, March 2018. Session Gynecologic Oncology Proceedings: F-098, 2018.
- 5. Robertson L, Fletcher NM, Saed GM. L-Alanyl-L-Glutamine Attenuates the Levels of Adhesion Phenotype Markers in Normal Fibroblasts Isolated From Human Peritoneum Under Hypoxic Conditions. 65<sup>th</sup> Annual Scientific Meeting for the Society for Reproductive Investigation, San Diego, CA, March 2018. Session Gynecology Proceedings: F-102, 2018.
- 6. Fletcher NM, Awonuga AO, Memaj I, Diamond MP, Saed GM. Interruption of MPO Binding to CD11B Selectively Kills Fibroblasts from Adhesion Tissues but not Normal Peritoneum. 73rd American Society for Reproductive Medicine Scientific Congress & Expo, San Antonio, TX, October-November 2017. Proceedings: P-264, 216, 2017. SRS In-Training Award for Research to NM Fletcher, PhD

Fletcher NM, Memaj I, Abusamaan MS, Juhani A, Al-Hendy A, Diamond MP, Saed **GM**. Oxidative Stress: A Key Regulator of Leiomyoma Cell Survival. 64th Annual Scientific Meeting for the Society for Reproductive Investigation, Orlando, FL, March 2017. Fertility and Sterility 24(1) Supplement: F-124, 208A, 2017.

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- Detti L, Fletcher NM, Saed GM, Uhlmann RA, Christiansen ME, Williams LJ. Anti-Mullerian Hormone (AMH) Regulates BRCA1 and BRCA2 Gene Expression in an Ovarian Cortex Transplantation Model. 72<sup>nd</sup> Annual Meeting of the American Society for Reproductive Medicine, Salt Lake City, UT, October 2016. Fertility and Sterility 106(3) Supplement: P-037, e120, 2016.
- Fletcher NM, Belotte J, Saed MG, Abusamaan MS, Diamond MP, Saed GM. Chemotherapy Induces a Genotype Switch in Key Antioxidant Enzymes: A Potential Mechanism of Chemoresistance in Epithelial Ovarian Cancer Cells. 63rd Annual Meeting of the Society for Reproductive Investigation, Montreal, Quebec, Canada, March Reproductive Sciences 23(1) Supplement: F-248, 262-263A, 2016.
- Detti L, Fletcher NM, Uhlmann RA, Belotte J, Williams LJ, Saed GM. Exposure to Recombinant Anti-Mullerian Hormone (AMH) Downregulates Ovarian Follicle Cells' Stemness Potential in Fresh and Vitrified/Thaw Ovarian Cortex, 63rd Annual Meeting of the Society for Reproductive Investigation, Montreal, Quebec, Canada, March 16-19, 2016. Reproductive Sciences 23(1) Supplement: T-257, 180A, 2016.
- Nusrat O, Belotte J, Fletcher NM, Saed MG, Diamond MP, Saed GM. Chemoresistant Ovarian Cancer Cells Manifest Lower Vascular Endothelial Growth Factor and Hypoxia Inducible Factor-1a: A Potential Survival Mechanism. 63rd Annual Meeting of the Society for Reproductive Investigation, Montreal, Quebec, Canada, March 16-19, 2016. Reproductive Sciences 23(1) Supplement: T-250, 178A, 2016.
- Fletcher NM, Neubauer BR, Saed MG, Abu-Soud HM, Saed GM. 2,4-Dinitrophenol Induced Cell Death of Ovarian Cancer Stem Cells. 62<sup>nd</sup> Annual Meeting of the Society for Reproductive Investigation, San Francisco, CA, March 2015. Reproductive Sciences 22(1) Supplement: S-003, 299A, 2015.
- 13. Fletcher NM, Neubauer BR, Saed MG, Diamond MP, Abu-Soud HM, Saed GM. Postoperative Adhesion Development is Controlled by Mechanisms Emanating from a Hypoxia-Induced Genotype Switch in Nicotinamide Adenine Dinucleotide Phosphate Oxidase Through the Up-Regulation of Cytidine Deaminase. 62<sup>nd</sup> Annual Meeting of the Society for Reproductive Investigation, San Francisco, CA, March 2015. Reproductive Sciences 22(1) Supplement: F-042, 218A, 2015.
- 14. Detti L, Williams LJ, Fletcher NM, Saed GM. Anti-Müllerian Hormone (AMH) May Inhibit Oocyte Maturation and Follicular Vascularization in Human Ovarian Cortex. Proceedings of the 71st Annual Meeting of the American Society for Reproductive Medicine, Baltimore, MD, October 2015. Fertility and Sterility 104(3) Supplement: P91, e136, 2015.

Fletcher NM, Saed MG, Neubauer BR, Abusamaan MS, Al-Hendy A, Diamond MP, 15. Berman JM, Saed GM. Uterine Fibroids Are Characterized by An Altered Redox Balance, Favoring a Pro-Oxidant State. 71st Annual Meeting of the American Society for Reproductive Medicine, Baltimore, MD, October 2015. Fertility and Sterility 104(3) Supplement: P-115, e145, 2015.

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- 16. Fletcher NM, Saed MG, Neubauer BR, Abu-Soud HM, Awonuga A, Diamond MP, Saed Shifting Anaerobic to Aerobic Metabolism Stimulates Apoptosis in Adhesion Fibroblasts Through the Modulation of the Cellular Redox Homeostasis. 71st Annual Meeting of the American Society for Reproductive Medicine, Baltimore, MD, October 2015. Fertility and Sterility 104(3) Supplement: P-215, e179, 2015.
- Abusamaan MS, Fletcher NM, Saed MG, Al-Hendy A, Diamond MP, Berman JM, Saed 17. GM. Myeloperoxidase Serves As A Redox Switch That Regulates Apoptosis In Human Leiomyomas. 71st Annual Meeting of the American Society for Reproductive Medicine, Baltimore, MD, October 2015. Fertility and Sterility 104(3) Supplement: P-113, e145, 2015.
- 18. Fletcher NM, Detti L, Neubauer BR, Saed MG, Diamond MP, Abuzeid MI, Saed GM. Altered Redox State in the Endometrium of Patients Undergoing Ovarian Stimulation for Assisted Reproduction Technology. Proceedings of the 70<sup>th</sup> Annual Meeting of the American Society for Reproductive Medicine, Honolulu, HI, October 2014. Fertility and Sterility 102(35) Supplement: P-426, e279, 2014.
- 19. Belotte J, Fletcher NM, Diamond MP, Saed GM. Sox2 Gene Amplification Impacts Survival in Serous Epithelial Ovarian Cancer. 61st Annual Meeting of the Society for Investigation, Florence, Italy, March 2014. Reproductive Sciences 21(3) Supplement: T-219, 204A, 2014.
- Saed GM, Fletcher NM, Belotte J, Levin NK, Simon MS, Abu-Soud HM, Tainsky MA, 20. Diamond. SNPs in Key Oxidants and Antioxidants Are Associated with Increased Risk Serve as Potential Targets for Ovarian Cancer. 61st Annual Meeting of the Society for Gynecologic Investigation, Florence, Italy, March 2014. Reproductive Sciences 21(3) Supplement: T-249, 213A, 2014.
- Diamond MP, Fletcher NM, Saed MG, Abu-Soud HM, Al-Hendy A, Saed GM. Fibroids 21. Manifest Oxidative Stress As Compared to Normal Myometrium. 42<sup>nd</sup> Annual AAGL Global Congress of Minimally Invasive Gynecology, Washington, DC, November 2013. The Journal of Minimally Invasive Gynecology 20(6) Suppl: S19, 2013.
- Diamond MP, Fletcher NM, Abuanzeh S, Saed MG, Saed GM. 22. Creation and Persistence of the Adhesion Phenotype: The Role of NOXs in Creating Oxidative 42<sup>nd</sup> Annual AAGL Global Congress of Minimally Invasive Gynecology, Washington, DC, November 2013.
- Fletcher NM, Saed MG, Abu-Soud HM, Al-Hendy A, Diamond MP, Saed GM. Distinct 23. Oxidative Stress Profile in Uterine Fibroids Versus Adjacent Myometrium. Conjoint Meeting of the International Federation of Fertility Societies and the 69<sup>th</sup> American Society for Reproductive Medicine, Boston, MA, October 2013. Fertility and Sterility 100(3) Suppl: S34, 2013.

24. Fletcher NM, Abuanzeh S, Saed MG, Abu-Soud HM, Diamond MP, Saed GM. Postoperative Adhesion is Characterized by a Unique Oxidative Stress Profile Which is Responsible for Creation and Persistence of the Adhesion Phenotype. Conjoint Meeting of the International Federation of Fertility Societies and the 69th American Society for Reproductive Medicine, Boston, MA, October 2013. Fertility and Sterility 100(3) Suppl: S31, 2013.

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- 25. Thakur M, Imudia AN, Shavell VI, Singh M, Diamond MP, Awonuga AO, Saed GM. Should Body Mass Index Influence the Dose of hCG for Ovulation Induction After Superovulation in IVF/ICSI cycles? 68th Annual Meeting of the American Society for Reproductive Medicine, San Diego, CA, October 2012. Fertility and Sterility 98(3) Suppl: P-542, S271, 2012.
- Fletcher NM, Al-Hendy A, Diamond M, Saed GM. Uterine Fibroids Are Characterized 26. by an Impaired Antioxidant Cellular System: Potential Role of Hypoxia in the Pathophysiology of Fibroids. 68<sup>th</sup> Annual Meeting of the American Society for Reproductive Medicine, San Diego, CA, October 2012. Fertility and Sterility 98(3) Suppl: P-403, S231, 2012.
- 27. Detti L, Uhlmann RA, Fletcher NM, Diamond MP, Saed GM. Endometrial Thyroid and Vitamin D Signaling Pathways during Ovarian Stimulation for Assisted Reproductive Technology (ART). 68th Annual Meeting of the American Society for Reproductive Medicine, San Diego, CA, October 2012. Fertility and Sterility 98(3) Suppl: P-384, S225, 2012.
- 28. Fletcher NM, Belotte J, Diamond MP, Saed GM. Dicholoroacetate Increases Sensitivity to Chemotherapy Treatment of Epithelial Ovarian Cancer Cells. 59th Annual Meeting of the Society for Gynecologic Investigation, San Diego, CA, March 2012. Reproductive Sciences 19(3) Suppl: S-065, 354A, 2012.
- 29. Banerjee J, Maitra D, Shaeib F, Saed GM, Diamond MP, Abu-Soud HM. Melatonin Prevents Hypochlorous Acid Induced Alteration of the Metaphase-II Mouse Oocyte Microtubule and Chromosomal Structure. 59th Annual Meeting of the Society for Gynecologic Investigation, San Diego, CA, March 2012. Reproductive Sciences 19(3) Suppl: E-212, 289A, 2012.
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- 31. Nair S, Saed GM, Atta HM, Diamond M, Al-Hendy A. Gene Therapy of Abdominal/Pelvic Post-Operative Adhesions: Targeting Adenovirus towards Human Peritoneal Adhesion Cells. 59th Annual Meeting of the Society for Gynecologic Investigation, San Diego, CA, March 2012. Reproductive Sciences 19(3) Suppl: T-066, 141A, 2012
- 32. Banerjee J, Maitra D, Shaeib F, Saed GM, Diamond MP, Abu-Soud H. Role of Melatonin in Preventing Hypochlorous Acid Induced Alterations in Microtubule and Chromosomal Structure in Metaphase-II Mouse Oocytes In Vitro. 67th Annual Meeting of the American Society for Reproductive Medicine, Orlando, FL, October 2011. Fertility and Sterility (Suppl 1): P-450, 2011.

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- 35. Nair S. Saed G. Nwaobasi N. Atta H. Al-Hendy A. Towards Gene Therapy of Pelvic Post-Operative Adhesions: Targeting Adenovirus Towards Human Adhesion Cells. 67th Annual Meeting of the American Society for Reproductive Medicine, Orlando, FL, October 2011. Fertility and Sterlity (Suppl 1): P-111, 2011.
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- 44. Saed GM, Hall DT, Omar MW, Shavell VI, Fletcher NM, Diamond MP. Regulation of metabolic activity of peritoneal fibroblasts by dichloroacetate provides a potential target for interventions to reduce postoperative adhesions. 65th Annual Meeting of the American Society for Reproductive Medicine, Atlanta, GA, October 2009. Fertility and Sterility 90(Suppl. 1): P127, October 2009.
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- 13. Belotte J, Fletcher NM, Saed MG, Neubauer BR, Saed GM. RAD21 gene amplification impacts survival in serous epithelial ovarian cancer. 4th Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014. Program and Abstracts: #P-04, pg. 17, 2014.
- 14. Belotte J, Fletcher NM, Nusrat O, Saed MG, Neubauer BR, Abu-Soud HM, Saed GM. Superoxide dismutase significantly delayed the development of cisplatin resistance in epithelial ovarian cancer cells. 4th Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014. Program and Abstracts: #P-03, pg. 16, 2014.
- 15. Awonuga AO, Fletcher NM, Belotte J, Diamond MP, Saed GM. The in-vivo effects of superoxide dismutase on the incidence and severity of postoperative adhesion development. 4th Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014. Program and Abstracts: #P-02, pg. 15, 2014.
- Saed GM. The role of oxidative stress in the pathogenesis of pro-fibrotic gynecologic 16. disorders. 4th Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014. Program and Abstracts: #O-13, pg. 13, 2014.
- Najafi T, Goud AP, Goud PT, Saed GM, Gonik B, Abu-Soud HM. Release of substrates, 17. cofactors, and products of nitric oxide synthase are altered during oocyte aging. 4th Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014. Program and Abstracts: #O-12, pg. 12, 2014.
- Shaeib F, Khan SN, Ali I, Dai J, Drewlo S, Saed GM, Abu-Soud HM. The impact of 18. myeloperoxidase on metaphase II mouse oocyte quality. 4th Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014. Program and Abstracts: #O-11, pg. 11, 2014.
- 19. Fletcher NM, Saed GM. Differential expression of glutathione peroxidase and glutathione reductase in chemoresistant epithelial ovarian cancer cells. 4th Annual Research

- Symposium of the Michigan Alliance for Reproductive Technologies and Science (MARTS), University of Michigan, Ann Arbor, MI, May 2013.
- 20. Shaeib F, Banerjee J, Thakur M, Saed MG, Diamond MP, Saed GM, Abu-Soud HM. Confocal 3-dimensional reconstruction can serve as a useful tool to quantify oxidative stress induced oocyte spindle damage. 3rd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 15, 2013. Program and Abstracts: #35, 2013.

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- 21. Fletcher NM, Saed GM. Differential expression of glutathione peroxidase and glutathione reductase in chemoresistant epithelial ovarian cancer cells. 3rd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2013: Program and Abstracts: #26, 2013.
- 22. Saed GM. Investigation of the role of oxidative stress in the pathophysiology of gynecologic fibrotic disorders including postoperative adhesions, fibroids, and endometriosis as well as ovarian cancer. 3rd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2013. Program and Abstracts: #20, 2013.
- 23. Belotte J, Mitchell A, Belotte J, Saed GM. Sox2 gene copy number alteration (CAN) significantly impact overall survival (OS) in serous epithelial ovarian cancer. 3<sup>rd</sup> Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2013. Program and Abstracts: #6, 2013.
- 24. Belotte J, Fletcher NM, Abuanzeh S, Levin NK, Simon NS, Diamond MP, Abu-Soud HM, Tainsky MA, Saed GM. A novel association between a catalase single nucleotide polymorphism and increased risk of ovarian cancer. 3rd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2013. Program and Abstracts: #4, 2013.
- 25. Awonuga AO, King NM, Belotte J, Abuanzeh S, Diamond MP, Saed GM. The in vitro effects of superoxide dismutase on the incidence and severity of post-operative adhesion development after cecal abrasion. 3rd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2013. Program and Abstracts: #3, 2013.
- 26. Shavell VI, Fletcher NM, Abu-Soud HM, Diamond MP, Saed GM, Detti L. Superoxide dismutase levels are elevated in the peri-implantation endometrium in women undergoing ovarian stimulation. 3rd Annual Michigan Alliance for Reproductive Technologies and Science Research Symposium, Wayne State University School of Medicine, Detroit, MI, May 2012. Proceedings and Abstracts: #15, 2012.
- 27. Maitra D, Abdulhamid I, Saed GM, Diamond MP, Pennathur S, Abu-Soud HM. Fluorescent heme degradation products in Sickle cell disease: role of hypochlorous acid

- 3<sup>rd</sup> Annual Michigan Alliance for Reproductive in hemoglobin destruction. Technologies and Science Research Symposium, Wayne State University School of Medicine, Detroit, MI, May 2012. Proceedings and Abstracts: #12, 2012.
- 28. Maitra D. Abdulridha RM. Byun J. Souza CEA. Baneriee J. Andreana PR. Diamond MP. Saed GM, Pennathur S, Abu-Soud. The reaction of HOCl and cyanocobalamin: corrin destruction and the liberation of cyanogens chloride. 3rd Annual Michigan Alliance for Reproductive Technologies and Science Research Symposium, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #11, 2012.

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- Fletcher NM, Belotte J, Diamond MP, Saed GM. Dicholoroacetate increases sensitivity to 29. chemotherapy treatment of epithelial ovarian cancer cells. 3rd Annual Michigan Alliance for Reproductive Technologies and Science Research Symposium, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #8, 2012.
- 30. Belotte J, Fletcher NM, Diamond MP, Saed GM. The role of oxidative stress in the development of cisplatin resistance in epithelial ovarian cancer. 3rd Annual Michigan Alliance for Reproductive Technologies and Science Research Symposium, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #6, 2012.
- 31. Banerjee J, Maitra D, Shaeib F, **Saed GM**, Diamond MP, Abu-Soud HM. Melatonin prevents hypochlorous acid induced alteration of the metaphase-II mouse oocyte microtubule and chromosomal structure. 3rd Annual Michigan Alliance for Reproductive Technologies and Science Research Symposium, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #5, 2012.
- 32. Maitra D, Shaeib F, Abdulridha RM, Souza CEA, Saed GM, Abu-Soud HM. Modulation of myeloperoxidase activity by self-generated hypochlorous acid. 3<sup>rd</sup> Annual Michigan Alliance for Reproductive Technologies and Science Research Symposium, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #1, 2012.
- 33. Maitra D, Shaeib F, Abdulridha RM, Souza CEA, Saed GM, Abu-Soud HM. Modulation of myeloperoxidase activity by self-generated hypochlorous acid. 2<sup>nd</sup> Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #32, 2012.
- Fletcher NM, Belotte J, Diamond MP, Saed GM. Dicholoroacetate increases 34. sensitivity to chemotherapy treatment of epithelial ovarian cancer cells. 2<sup>nd</sup> Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #31, 2012.

35. Belotte J, Fletcher NM, Diamond MP, Saed GM. The role of oxidative stress in the development of cisplatin resistance in epithelial ovarian cancer. 2<sup>nd</sup> Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #29, 2012.

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- 36. Banerjee J, Maitra D, Shaeib F, Saed GM, Diamond MP, Abu-Soud HM. Melatonin prevents hypochlorous acid induced alteration of the metaphase-II mouse oocyte microtubule and chromosomal structure. 2<sup>nd</sup> Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #25, 2012.
- 37. Maitra D, Abdulridha RM, Byun J, Souza CEA, Banerjee J, Andreana PR, Diamond MP, Saed GM, Pennathur S, Abu-Soud HM. The reaction of HoCl and cyanocobalamin: corrin destruction and the liberation of cyanogens chloride. 2<sup>nd</sup> Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #23, 2012.
- 38. Shavell VI, Fletcher NM, Abu-Soud HM, Diamond MP, Saed GM, Detti LL. Superoxide dismutase levels are elevated in the peri-implantation endometrium in women undergoing ovarian stimulation. 2<sup>nd</sup> Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Programs and Abstracts: #17, 2012.
- 39. Maitra D, Abdulhamid I, Saed GM, Diamond MP, Pennathur S, Abu-Soud HM. Fluorescent heme degradation products in sickle cell disease: role of hypochlorous acid in hemoglobin destruction. 2nd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Programs and Abstracts: #16. 2012.
- 40. Saed GM. Investigation of the role of oxidative stress in the pathophysiology of gynecologic fibrotic disorders including postoperative adhesions, fibroids, and endometriosis as well as ovarian cancer. 2<sup>nd</sup> Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #9, 2012.
- 41. Shavell VI, Fletcher NM, Jiang ZL, Saed GM, Diamond MP. Coupling oxidative phosphorylation with 2,4-dinitrophenol promotes development of the adhesion phenotype. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #26, 2011.

- 42. **Saed GM**. The role of oxidative stress in the pathophysiology of gynecologic fibrotic disorders including postoperative adhesions, fibroids, and endometriosis, as well as ovarian cancer. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #24, 2011.
- 43. Maitra D, Shaeib F, Diamond MP, Saed GM, Abu-Soud HM. Melatonin can attenuate HOCI mediated hemolysis, free iron release and heme degradation from hemoglobin. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #19, 2011.
- 44. Maitra D, Byun J, Andreana PR, Abdulhamid I, Diamond MP, Saed GM, Pennathur S, Abu-Soud HM. Reaction of hemoglobin with HOCI: possible link between free iron accumulation and oxidative stress. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #18, 2011.
- 45. Fletcher NM, Jiang ZL, Levin NK, Abu-Soud HM, Munkarah AR, Tainsky MA, Diamond MP, Saed GM. Positive correlation between serum myeloperoxidase and free iron levels with stage of ovarian cancer: potential biomarkers for early detection and prognosis of cancer. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #13, 2011.
- 46. Banerjee J, Maitra D, Shaeib F, Saed GM, Diamond MP, Abu-Soud HM. Role of melatonin in preventing hypochlorous acid induced alterations in microtubule and chromosomal structure in metaphase-II mouse oocytes in vitro. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #7, 2011.
- 47. Diamond MP, Saed GM. Reduction of postoperative adhesions. Catalyzing Collaboration between Industry and Academic in the Life Sciences – Women's Health Medicine: Part I, Therapeutic Strategies Meeting, Illinois Science and Technology Park, Skokie. IL. June 2007. Proceedings 2007.

## **Invited Lectures/Presentations**

## International/National

- Targeting Integrin αV/β1 Receptor Manifests Intriguing Anti-Tumor Effects in 1. Sensitive and Chemoresistant Ovarian Cancer Cells: Potential Therapeutic Target. 64th Annual Scientific Meeting of the Society for Reproductive Investigation, Orlando, FL, March 2017.
- 2. The Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer. University of Jordan, Amman, Jordan, July 2017.

3. Novel Innovative Targets for Ovarian Cancer Therapy. King Hussein Cancer Center. Amman, Jordan, July 2017.

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- 4. The Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer. King Hussein Cancer Center, Amman, Jordan, November 2016.
- 5. The Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer. University of Jordan, Amman, Jordan, November 2016.
- 6. New Insights for Ovarian Cancer Screening. 4th International Conference of the Jordanian Society of Pathology and Laboratory Medicine. In collaboration with the Arabic Division of the International Academy of Pathology, Amman, Jordan, April 2011.
- 7. Updates in Oxidative Stress and Ovarian Cancer. Modern Technology Application in Pathology Conference, Amman, Jordan, July 22 – August 1, 2010.
- 8. The Role of p53 in the Pathogenesis of Keloids. International Meeting on Mechanisms Involved in Tissue Repair and Fibrosis: Role of the Microfibroblast (Differentiation and Apoptosis), Lyon, France, December 1997.

# Local/Regional

- The Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer. Joint Annual 1. Reproductive Sciences Retreat, Departments of Obstetrics and Gynecology, Wayne State University School of Medicine and The University of Toronto; and Annual Michigan Alliance for Reproductive Technologies and Sciences (MARTS) Meeting at Wayne State University, Detroit, MI, October 2017. Retreat
- 2. Invited Guest Speaker. Tumor Microenvironment Section, Karmanos Cancer Center, Detroit Medical Center/Wayne State University School of Medicine, Detroit, MI, June 2016.
- 3. Molecular Biological Procedures. C.S. Mott Center for Human Growth and Development. Division of Reproductive Endocrinology and Infertility Laboratory Techniques Summer Course, Wayne State University School of Medicine, Detroit, MI, September 2015.
- New Insights into Pathogenesis of Ovarian Cancer. The C.S. Mott Center Summer 4. Reproductive Sciences Technology Course, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, July 2014.
- 5. The Role of Oxidative Stress in the Pathogenesis of Pro-Fibrotic Gynecologic Disorders. 4<sup>th</sup> Annual Scientific Retreat, The C.S Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014.
- 7. Release of Substrates, Cofactors, and Products of Nitric Oxide Synthase Are Altered during Oocyte Aging. 4th Annual Scientific Retreat, The C.S Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014.
- 7. The Impact of Myeloperoxidase on Metaphase II Mouse Oocyte Quality. 4th Annual Scientific Retreat, The C.S Mott Center for Human Growth and Development, Department

- of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014. First Prize Award
- 8. Differential Expression of Glutathione Peroxidase and Glutathione Reductase in Chemoresistant Epithelial Ovarian Cancer Cells. The Michigan Alliance for Reproductive Technologies and Science (MARTS), Fourth Annual Research Symposium, University of Michigan, Ann Arbor, MI, May 2013.

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- 9. The Role of Oxidative Stress in the Pathophysiology of Gynecologic Fibrotic Disorders: Postoperative Adhesions, Fibroids, Endometriosis, and Ovarian Cancer. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011.
- 10. New Insights in Ovarian Cancer Screening. Department of Obstetrics and Gynecology Wayne Day: New Frontiers in the Treatment of Gynecologic Cancer, Wayne State University School of Medicine, Detroit, MI, December 2010.
- 11. Molecular Characterization of Adhesion and Peritoneal Fibroblasts. Adhesion Mini Symposium, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, March 2001.
- 12. Multiplex RT/PCR Technique, Concept and Application. Center for Biomedical Research, College of Art and Sciences, Oakland University, Rochester, MI, May 1999.
- 13. Techniques for Characterizing and Manipulating DNA from the Basis of Much of Modern Biomedical Research. Department of Chemistry, Oakland University, Rochester, MI, January 1999.
- 14. Bcl-2/Bax Ratio as a Measure of the Rate of Apoptosis in Keloid Fibroblasts. Oxford Biomedical Research Inc., Oxford, MI, January 1998.
- 15. PCR Techniques, Concepts and Applications. Howard Hughes Research Program, Oakland University, Rochester, MI, May 1998.
- 16. Multiplex RT/PCR Technique, Concept and Application. Center for Biomedical Research, College of Art and Sciences, Oakland University, Rochester, MI, May 1997.
- 17. Application of RT/PCR. Department of Chemistry, Oakland University, Rochester, MI, June 1994.

### Invited Seminars and Grand Rounds

- New Insights into the Pathogenesis of Post-Operative Adhesions Development. Department of Obstetrics and Gynecology Grand Rounds, Georgia Regents University, Augusta, GA, January 2017.
- 2. Novel Innovative Targets for Ovarian Cancer Therapy. Cancer Center Seminar, Georgia Regents University, Augusta, GA, January 2017.

- 3. The Role of Oxidative Stress in the Pathogenesis of Pro-Fibrotic Gynecologic Disorders. Augusta Research Day, Department of Obstetrics and Gynecology Grand Rounds, Georgia Regents University, Augusta, GA, June 2013.
- 4. Dichloroacetate Induces Apoptosis of Epithelial Ovarian Cancer Cells Through the Inhibition of Oxidative Stress Enzymes. SGI-SMFM Scientific Meetings Abstract Presentations, Department of Obstetrics and Gynecology Grand Rounds, Wayne State University School of Medicine, Detroit, MI, February 2010.
- 5. *PCR Techniques Concepts and Clinical Applications*. Clinical Fellows Seminar, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, March 2000.
- 6. The Role of p53 and Apoptosis in the Pathogenesis of Keloids. Department of Obstetrics and Gynecology Grand Rounds, Wayne State University School of Medicine, Detroit, MI, July 1998.